



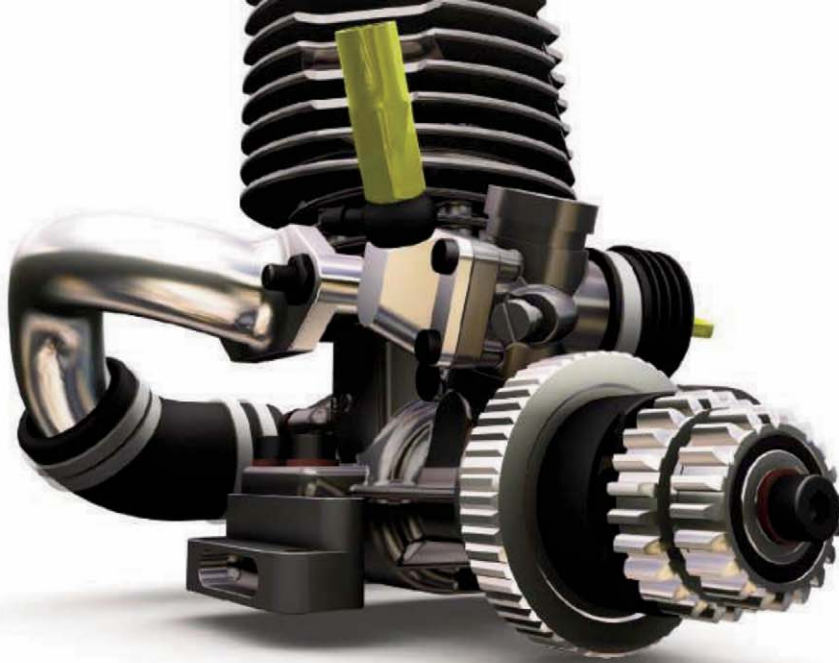
Science

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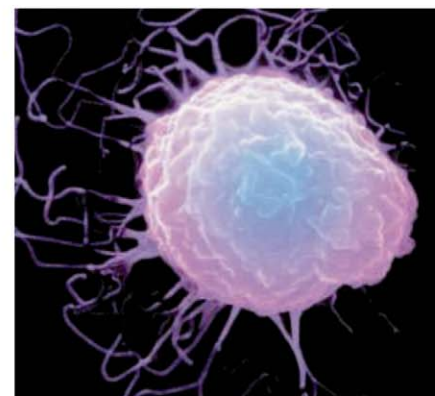
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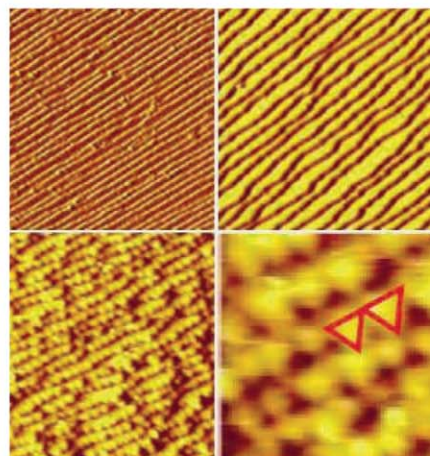
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Dark Matter Search Results from the CDMS II Experiment

The CDMS II Collaboration

Details of possible, but unlikely, detection events produced by dark matter are reported.
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A Stratified Redox Model for the Ediacaran Ocean

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RESEARCH ARTICLE: ARD1 Stabilization of TSC2 Suppresses Tumorigenesis Through the mTOR Signaling Pathway

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RESEARCH ARTICLE: SUMOylation Mediates the Nuclear Translocation and Signaling of the IGF-1 Receptor

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PERSPECTIVE: Genetic Polymorphisms—A Cornerstone of Translational Biobehavioral Research

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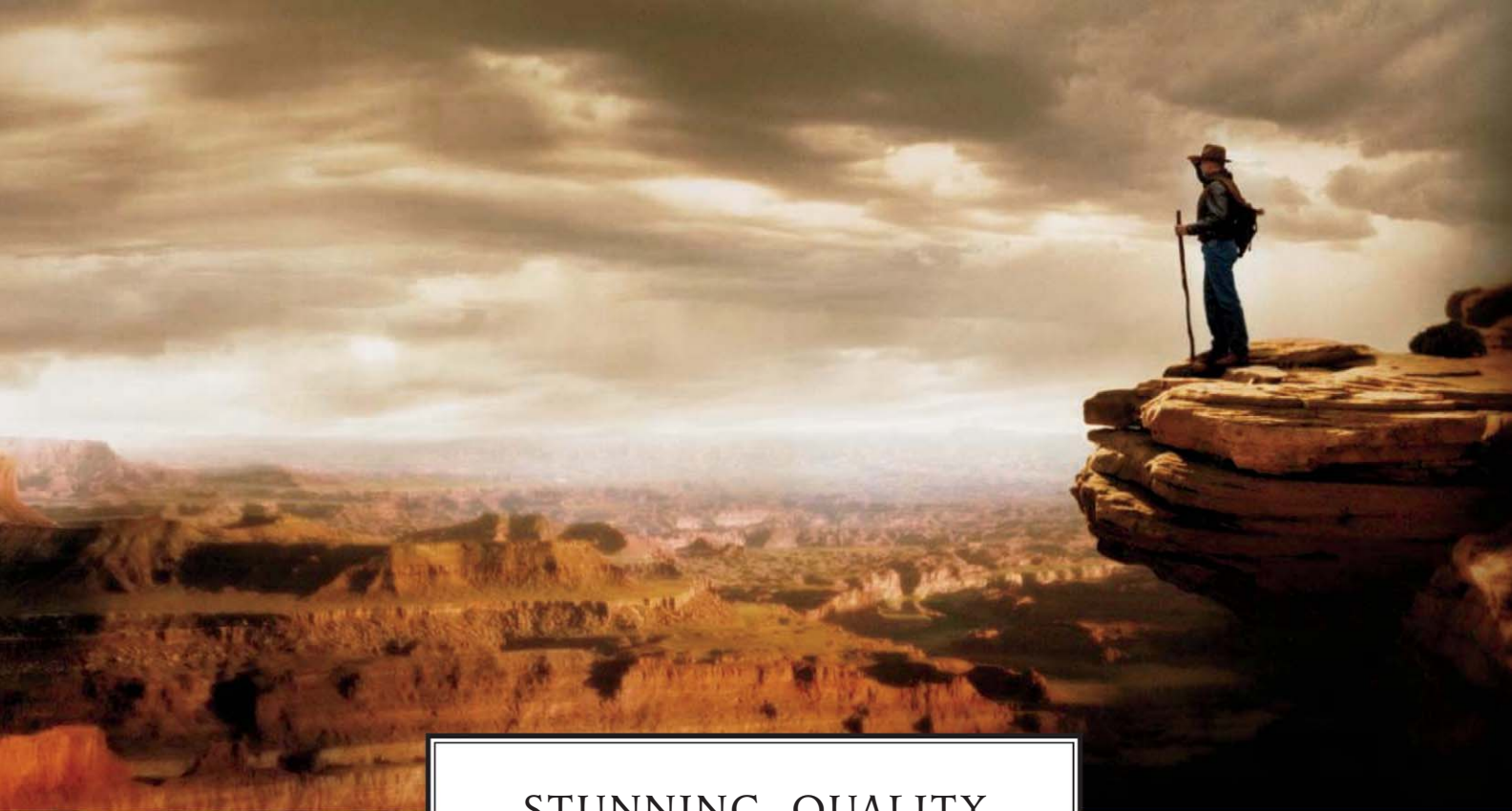
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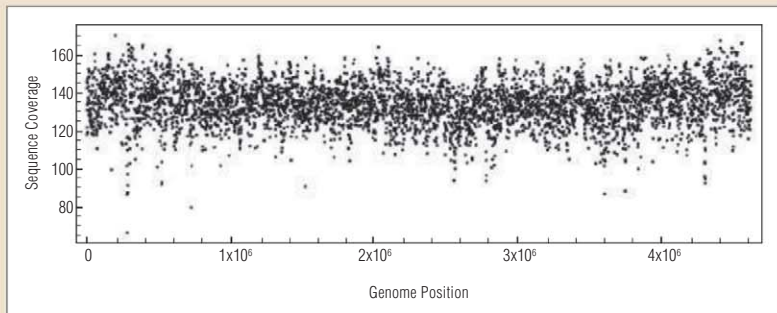


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<< Standing High

Sea-level rises and falls as Earth's giant ice sheets shrink and grow. It has been thought that sea level around 81,000 years ago—well into the last glacial period—was 15 to 20 meters below that of today and, thus, that the ice sheets were more extensive. **Dorale *et al.*** (p. 860; see the Perspective by **Edwards**) now challenge this view. A speleothem that has been intermittently submerged in a cave on the island of Mallorca was dated to show that, historically, sea level was more than a meter above its present height. This data implies that temperatures were as high as or higher than now, even though the concentration of CO₂ in the atmosphere was much lower.

Homing in on Hotspots

The clustering of recombination in the genome, around locations known as hotspots, is associated with specific DNA motifs. Now, using a variety of techniques, three studies implicate a chromatin-modifying protein, the histone-methyltransferase PRDM9, as a major factor involved in human hotspots (see the Perspective by **Cheung *et al.***). **Parvanov *et al.*** (p. 835, published online 31 December) mapped the locus in mice, and analyzed allelic variation in mice and humans, whereas **Myers *et al.*** (p. 876, published online 31 December) used a comparative analysis between human and chimpanzees to show that the recombination process leads to a self-destructive drive in which the very motifs that recruit hotspots are eliminated from our genome. **Baudat *et al.*** (p. 836, published online 31 December) took this analysis a step further to identify human allelic variants within Prdm9 that differed in the frequency at which they used hotspots. Furthermore, differential binding of this protein to different human alleles suggests that this protein interacts with specific DNA sequences. Thus, PDRM9 functions in the determination of recombination loci within the genome and may be a significant factor in the genomic differences between closely related species.

Superconducting Quantum Optics

The coherence properties of superconducting circuits enable them to be developed as qubits in quantum information processing applications. **Astafiev *et al.*** (p. 840) now show that these macroscopic superconducting devices also behave as artificial atoms and can exhibit quantum optical effects. The ability to fabricate and

integrate these superconducting devices in electronic circuitry may help toward developing a fully controlled quantum optics system on a chip.

Wave-Particle Duality

The dual-wave nature of particles is nowhere more evident than in a confined space, where standing waves are formed with wavelengths that depend on particle energy. This so-called quantum interference has been observed in nanostructures using surface probes such as scanning tunneling microscopy. Now, **Oka *et al.*** (p. 843) use the spin-polarized version of this technique to study spin-dependent quantum interference on a triangular nanoscale cobalt island deposited on a copper surface. They observe the modulation of the magnetization, with the pattern depending on the energy of the interfering electrons. The experimental results are in good agreement with simulations, which indicate that the magnetization at a given energy and position largely depends on which of two electron spin states present dominates.

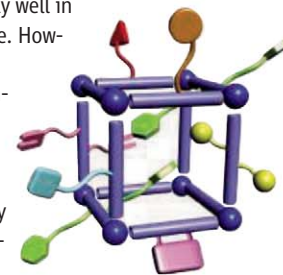
Colliding in the Cold

Chemical reactions occur through molecular collisions, which, in turn, are governed by the distributions of energy in each colliding partner. What happens when molecules are cooled so that they no longer have sufficient energy to collide? **Ospelkaus *et al.*** (p. 853; see the Perspective by **Hutson**) explored this question by preparing a laser-cooled sample of potassium rubidium (KRb) diatomics with barely any residual energy in any form (translational, rotational, vibrational, or electronic). By monitoring heat release over time, evidence was gathered for exothermic atom exchange reactivity through

quantum mechanical tunneling. As predicted by theory, these reactions were exquisitely sensitive to the molecular states, with rates changing by orders of magnitude on varying minor factors such as nuclear spin orientation.

Many Mixed Linkers in MOFs

Crystallization can separate different molecules because different molecules cannot generally be accommodated equally well in the same crystal lattice. However, in metal-organic framework (MOF) compounds, the organic linkers do not pack closely to other parts of the lattice, so it may be possible to mix several linkers that are derivatives of a parent compound with the same end groups. **Deng *et al.*** (p. 846) show that zinc-based MOFs can be made that mix 1,4-benzenedicarboxylate and up to eight of its derivatives in a random fashion. The effects of such mixing on porosity and absorption characteristics is nonlinear; in one case, a mixed-linker compound was four times better for selecting CO₂ versus CO compared with the best MOF bearing only one of the component linkers.



Salty Stretch

What happens at the molecular level when salt dissolves in water? Much of the data characterizing the geometry and dynamics of ion solvation

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shells has come from indirect observation of the surrounding water structure. Using a time domain Raman technique based on the interference of four ultrashort polarized light pulses, **Heisler and Meech** (p. 857) have now mapped directly the stretching vibrations associated with the weak hydrogen bonding interactions between bulk water molecules and chloride, bromide, or iodide ions.

Of Mice and Men

Just how closely must mouse models replicate the known features of human disorders to be accepted as useful for mechanistic and therapeutic studies? **Soliman et al.** (p. 863, published online 14 January) compared mice that vary only in their allelic composition at one position within the gene encoding brain-derived neurotrophic factor (BDNF) with humans exhibiting the same range of allelic variation. Individuals (mice and humans) carrying the allele that codes for a methionine-containing variant of BDNF retained a fearful response to a threatening stimulus even after its removal in comparison to those with the valine variant. Furthermore, in both cases, this linkage was mediated by diminished activity in the ventral-medial region of the prefrontal cortex. This deficit in extinction learning may contribute to differential responses to extinction-based therapies for anxiety disorders.



Decoding a Second-Messenger's Message

Biofilms are aggregates of bacteria on a surface often associated with increased resistance to antibiotics and stress. In *Vibrio cholerae*, the bacterial species that causes cholera, biofilm formation is promoted by the bacterial second-messenger cyclic diguanylate (c-di-GMP) and involves the transcription regulator, VpsT. **Krasteva et al.** (p. 866) show that VpsT is itself a receptor for c-di-GMP and that binding of the small signaling molecule promotes VpsT dimerization, which is required for DNA recognition and transcriptional regulation. As well as activating components of the biofilm pathway, VpsT also down-regulates motility genes in a c-di-GMP-dependent manner.



DNA-less Evolution

Prions are proteinaceous infectious elements involved in a variety of neurodegenerative diseases, including scrapie in sheep and so-called mad cow disease in cattle. Now **Li et al.** (p. 869, published online 31 December) show that, when propagated in tissue culture cells, cloned prion populations become diverse by mutational events and can undergo selective amplification. Thus, even though devoid of a coding genome, prions, when propagated under a particular selection regime, can be subject to rapid evolution.

Viral Superspreaders

Viruses are thought to spread across a lawn of cells by an iterative process of infection, replication, and release. If this were the case, the rate of spread would be limited by the viral replication kinetics. Now, **Doceul et al.** (p. 873, published online 21 January; see the Perspective by **Pickup**) describe a spreading mechanism used by vaccinia virus that is not restricted by viral replication kinetics and that causes a dramatic acceleration of spread. Early after infection, vaccinia virus proteins A33 and A36 are expressed as a complex on the cell surface. This marks the cell as infected and causes superinfecting virions to be repelled by the formation of actin projections beneath the virus particle. Virions are repelled from infected cells repeatedly until an uninfected cell is reached and are thus pushed further away from the origin of infection to accelerate dissemination.

It's All About Self-Renewal

The *Lmo2* oncogene was identified as a contributing factor in human T cell acute lymphoblastic leukemia (T-ALL) nearly two decades ago, but the gene rose to prominence in 2003 when its inadvertent activation by a retroviral vector was shown to cause leukemia in two patients in a gene therapy trial. The cellular mechanism by which the gene product of *Lmo2*, a transcriptional regulator, induces T-ALL is poorly understood. Studying transgenic mice, **McCormack et al.** (p. 879, published online 21 January) now show that *Lmo2* confers self-renewal activity to committed T cells in the thymus without affecting their capacity for T cell differentiation. These self-renewing cells, which were detectable 8 months prior to the onset of overt leukemia in the mice, expressed genes in common with hematopoietic stem cells (HSCs), suggesting that *Lmo2* might reactivate an HSC-specific transcriptional program.

CREDIT: KRASTEVA ET AL.

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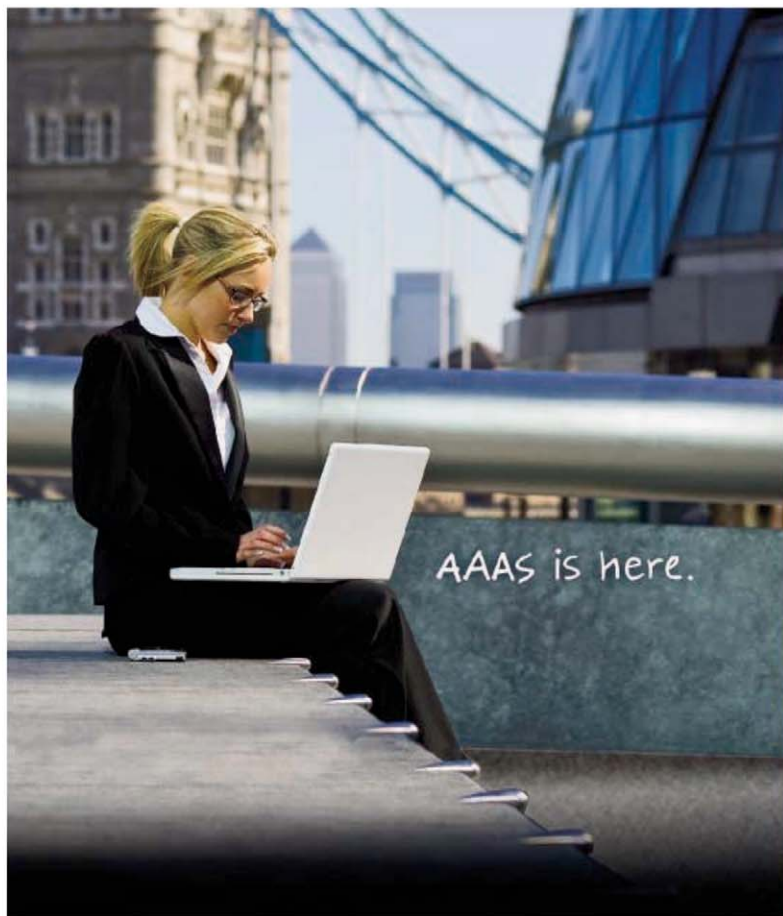
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David Baulcombe is Regius Professor of Botany and Royal Society Research Professor at the University of Cambridge. His research is on RNA silencing, epigenetics, and disease resistance in plants. Email: dcb40@cam.ac.uk

Reaping Benefits of Crop Research

IN 2009, FOR THE FIRST TIME SINCE THE 1950S AND THE EARLY STAGES OF THE GREEN REVOLUTION, food security was taken seriously by policy-makers. There was substantial output from the International Assessment of Agricultural Knowledge, Science and Technology for Development, and with studies by the U.S. National Academy of Sciences and a UK government Foresight group due this year, there is no sign that this renewed interest will fade. This revival follows assessments by the United Nations Food and Agriculture Organization and others that population growth, urbanization, climate change, and the availability of natural resources present a challenge to global food security. Somehow the world must produce 50 to 100% more food than at present under environmental constraints that have not applied in the past.

Although raw statistics indicate that enough food can be produced with existing technologies, the figures hide the inequity and unsustainability of current global food production. Inequity is evidenced by 1 billion hungry people. Unsustainability is a stark reality, because the highest levels of food crop productivity in many regions deplete stocks of nonrenewable resources, damage ecosystem services, and have a large carbon footprint (through carbon depletion of soils, fuel combustion, and the energy cost of fertilizer production). Thus, the challenge not only concerns the amount of food produced, it is also about equity, energy use, and sustainability.

Last year, a UK Royal Society working group, which I chaired, concluded in the report *Reaping the Benefits** that there are few opportunities to cultivate additional land without causing environmental damage. “Sustainable intensification” was proposed, in which biological sciences play a prominent role, calling for crop production that is resistant to stresses and disease; produces consistent yields using renewable inputs; avoids depletion of minerals, biodiversity, and natural capital; and protects ecosystem services.

Achieving these goals will require new research that integrates current practices in diverse agricultural systems with rapidly advancing research in genomics, systems biology, microbiology, and cell biology. Genome sequencing is particularly valuable because it facilitates exploitation of the vast untapped genetic variation in crops and crop relatives. An integrated research strategy will allow progressive refinement of existing crop varieties, so as to bolster pest and disease resistance, while developing varieties and crop management practices that use water and fertilizers efficiently. Radical changes also may be possible, such as perennial cereals, the widespread use of companion cropping with nitrogen-fixing legumes, asexual seed production to capture hybrid vigor, and even supercharging photosynthesis. Many of these innovations, some of which involve genetically modified crops, would allow high yield with lower inputs of water or fertilizer than in current industrial agriculture. Any gross yield reductions associated with sustainability innovations in these industrialized systems would be justified by reduced use of scarce or nonrenewable resources. On a global scale, such a decrease should be offset by increases in developing countries, where there is great scope to boost gross output because the current average yields are so low.

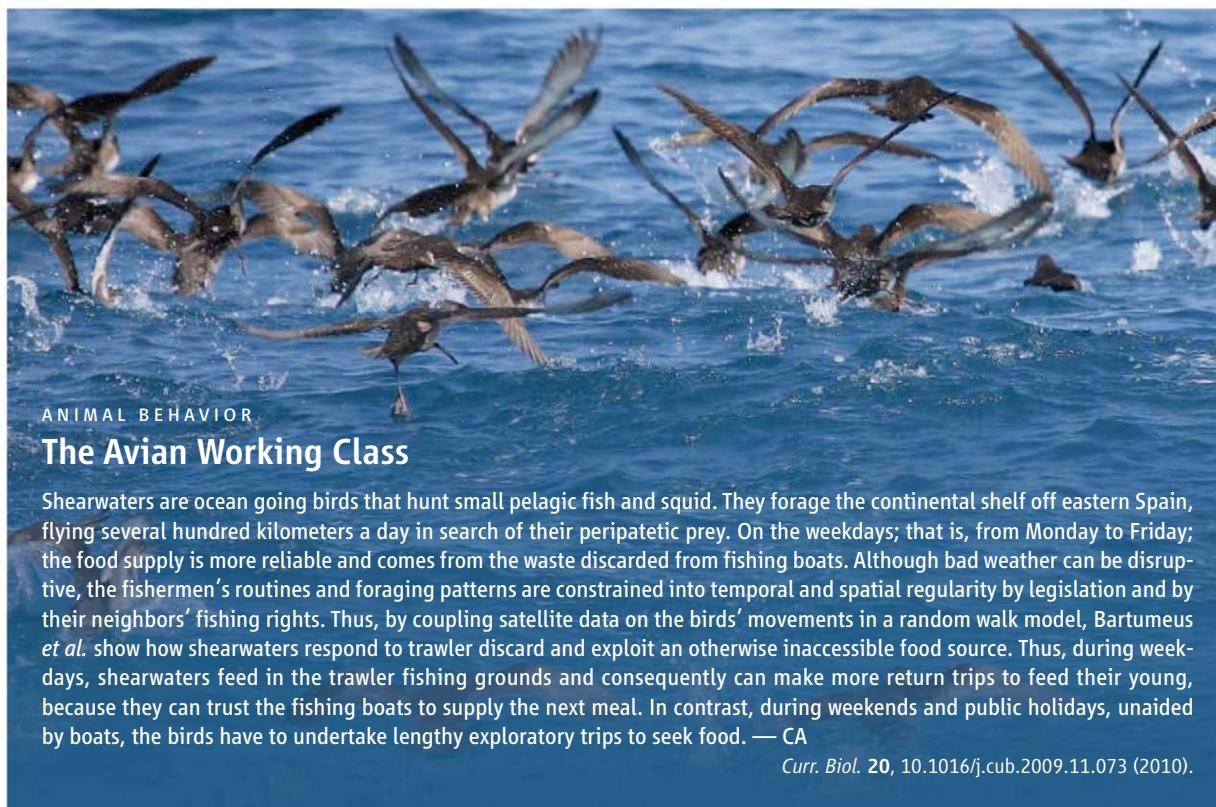
A critical factor, emphasized in the Royal Society report, is a major gap in skills and training. Scientists who can link practical applications related to crops with the latest developments in the life sciences are in very short supply. Molecular genetics has led to rapid progress in understanding crop plant biology, but unless universities rethink their strategies for training in all of the disciplines relevant to food crop science, there will be a continued shortage of appropriately trained scientists who can translate this progress into practical applications. Universities and funders should also internationalize training through collaborations with developing countries, so that modern science can be linked to practical needs in regions where there is great need for technological advance.

— David Baulcombe

10.1126/science.1186705



*<http://royalsociety.org/Reapingthebenefits>.



ANIMAL BEHAVIOR

The Avian Working Class

Shearwaters are ocean going birds that hunt small pelagic fish and squid. They forage the continental shelf off eastern Spain, flying several hundred kilometers a day in search of their peripatetic prey. On the weekdays; that is, from Monday to Friday; the food supply is more reliable and comes from the waste discarded from fishing boats. Although bad weather can be disruptive, the fishermen's routines and foraging patterns are constrained into temporal and spatial regularity by legislation and by their neighbors' fishing rights. Thus, by coupling satellite data on the birds' movements in a random walk model, Bartumeus *et al.* show how shearwaters respond to trawler discard and exploit an otherwise inaccessible food source. Thus, during weekdays, shearwaters feed in the trawler fishing grounds and consequently can make more return trips to feed their young, because they can trust the fishing boats to supply the next meal. In contrast, during weekends and public holidays, unaided by boats, the birds have to undertake lengthy exploratory trips to seek food. — CA

Curr. Biol. **20**, 10.1016/j.cub.2009.11.073 (2010).

PHYSICS

Factoring in Noise

Quantum dots are semiconducting nanostructures often referred to as "artificial atoms" because of the discreteness of their energy levels. However, unlike real atoms, quantum dots of a given elemental formula are not all created equal: A typical manufacturing process inevitably results in dots that vary in shape and size, leading to differences in energy levels and other properties. Thus, the full ensemble of quantum dots must be carefully characterized before the potential use of its constituents in spintronics and quantum information applications. One of the most important properties in these contexts is the current carriers' response to external magnetic fields, quantified by the so-called Landé or *g* factor. The *g* factor is usually measured through optical pump-probe studies. Now, Crooker *et al.* have analyzed the weak spin noise signature in (In,Ga)As/GaAs quantum dots using sophisticated power spectral averaging to extract the response of both negative (electron) and positive (hole) carriers. The applied magnetic field causes the carriers' spins to precess and centers the spin fluctuation spectrum at the associated Larmor frequency, proportional to the

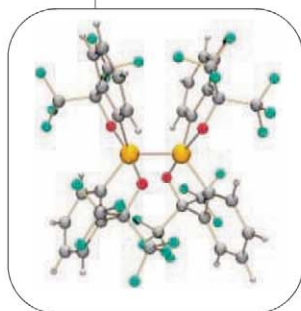
g factor. The study reveals that the hole *g* factor is highly anisotropic and that within the sample this anisotropy varies monotonically with the quantum dot confining energy. — JS

Phys. Rev. Lett. **104**, 36601 (2010).

CHEMISTRY

Five on Five

In most molecular contexts, silicon behaves like carbon in forming covalent bonds to four neighboring atoms. Kano *et al.* have now coaxed the element into a more crowded motif. Specifically,



by using lithium as an electron source, the authors reduced two four-coordinate silicon centers and brought them together to form a bond. The resulting dianion, characterized in solution as well as the solid state, proved remarkably stable, even persisting for days in boiling water. Protonation with acid liberated a substituent at each silicon, forming a product with more conventional four-coordinate centers, but the process was efficiently reversed on treatment

with a strong base. The authors attribute the stability of the unusual five-coordinate bonding arrangement partly to the electron-withdrawing character of the surrounding substituents (benzyl alcohol derivatives bearing trifluoromethyl groups). Theoretical calculations supported a bond order between silicons approaching 1 and suggested that the silicon centers themselves bore positive charges, despite the overall dual negative charge of the complex. — JSY

Nat. Chem. **2**, 112 (2010).

MATERIALS SCIENCE

Stringing DNA Along

The high base-pairing fidelity of DNA makes the biopolymer a powerfully versatile templating material for precise nanoscale fabrication. Unfortunately, it is costly to prepare long sequences and thus to direct structure over a long range. In contrast, synthetic block copolymers are well suited to creating periodic structures over long distances because of the microphase separation of the covalently linked blocks that ensues in a selective solvent. Carneiro *et al.* attached a dendritic oligo(ethylene glycol) (OEG) unit to one end of 10- to 20-base pair DNA oligomers. Hybridization with the complementary DNA strands then formed a

triblock, with dendritic units at either end that could assemble into long fibers when a selective solvent was added. The fibers extended for several micrometers and could further align into parallel rows. Fiber formation could be tuned or eliminated by changing the ratio of DNA to OEG or by changing the number of arms on the OEG. More complex structures could also be prepared by hybridizing three linking strands to three dendritic DNA strands, yielding a three-helix bundle that, despite the internal complexity, could still form long ordered strands. — MSL

J. Am. Chem. Soc. **132**, 679 (2010).

BIOPHYSICS

Molecular Yoga

The influence of tremendous advances in biological crystallography—most notably of membrane proteins and of large complexes of nucleic acids and proteins—has been profound. Perhaps too much so, for the glittering array of colorful macromolecules has tended to obscure the fact that they are constantly stretching, contracting, bending, or twisting.

Using molecular dynamics and normal mode analysis, Grinthal *et al.* illustrate the potential biological impact of these restless movements. Protein phosphatase 2A (PP2A) consists of a catalytic subunit (yellow), a regulatory subunit (green), and the PR65 scaffold (blue). This last component contains 15 repeats of a two-helix unit and adopts a curved solenoid shape. The lowest-frequency mode of the PP2A heterotrimer combines torsion and flexion of PR65, and the



effect is to repetitively open and close the catalytic site (red) located at the interface between the other two subunits. Tuning these motions either by transiently applying force or via mutation within the interhelix linkages would result in what might be called an elasto-steric regulation of enzyme activity. — GJC

Proc. Natl. Acad. Sci. U.S.A. **107**, 10.1073/pnas.0914073107 (2010).

IMMUNOLOGY

Gut Reactions Gone Awry

Crohn's disease is a debilitating autoimmune disease of the gastrointestinal tract. Genome-wide association studies have demonstrated strong links between Crohn's disease and polymorphisms in genes involved in microbial

recognition (*NOD2*) and autophagy (*ATG16L1*), which is a broad-spectrum intracellular degradation pathway. How microbial recognition and autophagy might intersect, however, has been unclear.

Travassos *et al.* and Cooney *et al.* have found that *NOD2* detection of bacterial peptidoglycans results in the recruitment of *ATG16L1* to sites of bacterial entry at the plasma membrane. This aids in the formation of autophagosomes, a process that promotes bacterial degradation and leads to the presentation of bacterial antigens to CD4⁺ T cells. Both groups then went on to connect these events to Crohn's disease. Using cells either from Crohn's disease patients expressing the disease-associated variants of *NOD2* or *ATG16L1* or from mice homozygous for a *NOD2* disease-associated variant, they observed deficits in *ATG16L1* localization to the plasma membrane, autophagy induction, antigen presentation, and bacterial clearance. Together, these studies suggest that bacterial persistence, due to impaired autophagic degradation, may be an important driver in the pathogenesis of Crohn's disease. — KLM

Nat. Immunol. **11**, 55; *Nat. Med.* **16**, 90 (2010).

BIOTECHNOLOGY

The Heart of Nanotechnology

Muscle cells in the heart must adopt specific orientations so that they can work together to produce strong contractile forces. The cells receive organizing cues from the surrounding extracellular matrix (ECM). In tissue culture, myocyte orientations are random in the absence of ECM, and large-scale patterning (on the order of 10 μm) of the substratum can instill an organized pattern of growth.

Kim *et al.* have used capillary lithography to create a synthetic extracellular matrix from polyethylene glycol hydrogel arrays, with grooves and ridges a few hundred nanometers tall and wide—a scale that may mimic the effect on cells of ECM fibrils in vivo. Although the rat ventricular myocytes sometimes spanned several ridges, they were sensitive to ridge spacing. The physical properties of the synthetic matrix influenced cell orientation, cell size, adhesion, and electrophysiological properties. Expression of the gap junction protein connexin 43, which supports intercellular communication, was also sensitive to variation in the spacing of the ridges. The authors note that the ability to synthesize such materials may be essential for engineering tissue-repair processes in medical applications. — LBR

Proc. Natl. Acad. Sci. U.S.A. **107**, 565 (2010).

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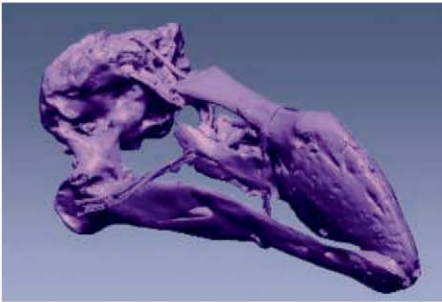
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Care for a Virtual Wishbone?

Birds' adaptations for flight are more than feather deep. Hollow, lightweight bones and a keeled sternum that anchors flight muscles also help them take to the skies. Now you can get a close look at bird skeletal specializations with Aves 3D, headed by evolutionary biologists Leon



Claessens of the College of the Holy Cross in Worcester, Massachusetts, and Scott Edwards of Harvard University.

The new database houses three-dimensional laser scans of bones from almost 100 living and extinct species. Rotate and zoom in on the breastbone of an American flamingo, the wishbone of a king penguin, or the skull of the defunct dodo (above). Register for free to download some models for further analysis at www.aves3D.org.

Harbors on the Move

More supersize ships are plying the high seas these days, and many ports are too shallow to handle them. So scientists at the Korea Advanced Institute of Science and Technology (KAIST) have come up with an alternative to expensive dredging operations: a mobile harbor.

Instead of deepening a port, "why shouldn't they send a harbor to the ship?" says KAIST Professor Kwak Byung Man. Last year, KAIST put \$22 million into designing the world's first



seagoing harbor. The 77-meter platform would use a robotic arm to join with a container ship anchored offshore and then offload cargo containers with a crane tipped with a "spreader" that stretches or shrinks in response to wave movements. "We thought of our hands that can reach out and grab an object even when we are in a rocking boat," says Kwak.

Kwak says a new shipping lane at the Panama Canal, to be completed in 2014, and mobile harbors will enable supersize ships to deliver containers to hitherto inaccessible ports, allowing shippers to bypass rails and trucks. Avoiding congested harbors such as Singapore's and risks of terrorism could also inspire buyers, says Kim Yong-Im, manager of project development at KAIST.

Last December, KAIST simulated the operation in a test pool (below). KAIST expects to be able to produce customized mobile harbors, priced between \$40 million and \$50 million, sometime in 2012.

Tangled Turkey Tale

Mesoamericans have been credited with introducing domesticated turkeys to North America sometime after 200 B.C.E. But a genetic study suggests that people in what is now the southwestern United States tamed turkeys on their own.

The history of the turkey, one of the few animals to be domesticated in the New World, has been complicated by wars, diseases, Spanish turkey traffic, and even 20th century wild turkey-release programs.

To untangle the turkey story, Brian Kemp, a molecular anthropologist at Washington State University, Pullman, and colleagues tested turkey DNA from a variety of sources: 38 southwestern archaeological sites dated from 200 B.C.E. to 1800 C.E., 10 museum specimens of extinct Mesoamerican wild turkeys, 12 grocery store turkeys, and almost 300 turkey sequences in the GenBank database.

The southwestern turkeys were only distantly related to the Mesoamerican birds, suggesting that the Pueblos didn't inherit their domesticated turkeys from the Mesoamericans, the team reported online last week in the *Proceedings of the National Academy of Sciences*. Furthermore, the Pueblos didn't go after local birds to tame, but preferred those from the eastern and midwestern United States—perhaps because of their superior feathers.



MOON OVER THE RUHR

The "largest moon on Earth" is attracting record numbers of visitors to Gasometer Oberhausen in Germany's Ruhr Valley. The 25-meter-diameter moon is suspended in the Gasometer's unusual exhibition space: a 100-meter-high tank that was built to store the gas produced by the region's iron and coke processing plants. The sculpture, created by photographer Wolfgang Volz, is based on high-resolution satellite images and mimics the moon's phases in a 5-minute show. The exhibition runs through the end of the year.

The finding validates a 1980 proposal by ethnozoologist Charmion McKusick of the former Southwest Bird Laboratory in Globe, Arizona, who based her analysis on turkey skeletal features. "We could not replicate [McKusick's] measurement studies, ... so we weren't persuaded" at the time, says Robin Lyle, a turkey researcher with the Crow Canyon Archaeological Center in Cortez, Colorado. But now "it's all beginning to make sense."



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PARTICLE PHYSICS

New Delay of Large Hadron Collider Might Not Keep Its Rival on the Job

The news in particle physics last week wasn't as surprising as the reaction to it. The world's highest-energy atom smasher, the Large Hadron Collider (LHC), will run at half its maximum energy through 2011 and not at all in 2012, officials at the European particle physics laboratory, CERN, near Geneva, Switzerland, announced. They had previously planned to run the beleaguered accelerator at 70% of maximum energy this year. The cut in energy reduces CERN physicists' chances of spying the long-sought Higgs boson—the hypothesized particle central to physicists' explanation of the origin of mass—before rivals at the Fermi National Accelerator Laboratory (Fermilab) in Batavia, Illinois, might spot it.

Curiously, though, Fermilab physicists did not immediately clamor to run their 27-year-old Tevatron collider for an extra year through 2012. That contrasts to last year, when in response to a delay to the LHC, Fermilab scientists pushed hard to run the Tevatron through 2011, a move the U.S. Department of Energy (DOE) supports (*Science*, 20 February 2009, p. 993). This time, Fermilab physicists say an extra year's worth of data might not be worth the expense. "It's not like we're rushing out and saying 'We want to run in 2012!'" says Fermilab's Dmitri Denisov, co-spokesperson for the 510-member team working with the D0 particle detector. "But we want to keep the possibility open."

CERN's new plan aims to further ensure the LHC's safety while amassing a useful amount of data, says Steve Myers, director of accelerators and technology at CERN. The

\$5.5 billion LHC is designed to blast protons into protons at an energy of 14 trillion electron-volts (TeV)—seven times the Tevatron's maximum. But CERN officials must keep the energy low to protect faulty electrical connections, or "interconnects," between the thousands of superconducting magnets that guide particles around the 27-kilometer subterranean ring. In September 2008, just 9 days after it first circulated particles, the LHC broke down when an interconnect melted, and researchers spent 14 months repairing the damage. CERN officials had planned to start running soon at 7 TeV and ramp up to 10 TeV this year. They have now scaled back

the energy to 7 TeV through next year.

The LHC will run until experimenters collect enough data—1 inverse femtobarn, in the units they use—to give them a shot at discovering new particles predicted by a theory called supersymmetry. It will then shut down for a year so workers can replace all of the roughly 10,000 interconnects, allowing the LHC to run at 14 TeV in 2013. "By doing it this way, we have the time needed to design the new interconnects in a thorough way and make sure it's done correctly," Myers says.

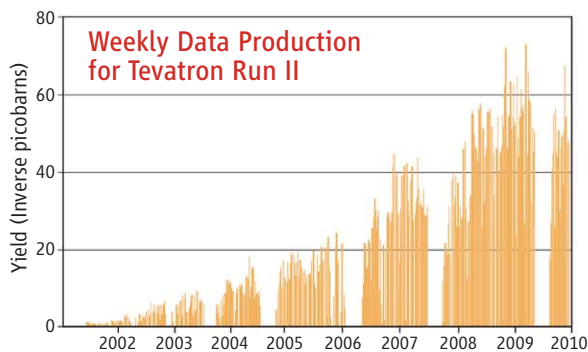
CERN physicists are pleased with the guarantee of 1 inverse femtobarn of data, says CERN's Guido Tonelli, spokesperson for the 3800-member team working the Compact Muon Solenoid particle detector. "Clearly, we would have preferred to run at a higher energy," he says, "but this is a real physics run in which we will be able to tackle a large part of our research program."

Initially, CERN physicists said the LHC would start in 2007 and that it made little sense to keep the redundant Tevatron running past 2008 (*Science*, 2 June 2006, p. 1302). Mishaps and delays at the LHC have given the Tevatron one reprieve after another. But physicists working on the older machine say they will soon face a problem of diminishing returns.

To continue to improve chances of spotting something new, Fermilab physicists need to steadily increase the accelerator's collision rate and double the size of their data set at regular intervals—about 2 years. But the Tevatron's collision rate has leveled off (see graph), and running in 2012 would likely add only another 25% to the 12 inverse femtobarns researchers expect to have by the end of 2011. It would also divert resources from neutrino experiments and other new projects, says Fermilab Director Pier Oddone.

Still, Fermilab physicists aren't ready to write off the Tevatron just yet. Forging ahead might make sense if the data start to show hints of the Higgs boson or some other new particle, Denisov says. The Tevatron would also be an option if the LHC suffers another catastrophe, Oddone says. "The real decision would then be, do you run the Tevatron for 3 more years?" he says. "I hope we don't get to that point." DOE won't have to make a decision on running the Tevatron in 2012 for several months.

—ADRIAN CHO



Leveling off. Throughout the past decade, Fermilab physicists doubled the rate at which Tevatron smashes particles roughly every 2 years. Now the machine's productivity has plateaued, so running another year would yield only marginally more data.

CREDITS: (DATA SOURCE) FERMILAB; (PHOTO) REIDAR HAHN/FERMILAB



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novelist

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INDIA

After Acrimonious Debate, India Rejects GM Eggplant

NEW DELHI—The standard bearer of the next food revolution in India was supposed to be an eggplant. Instead, the unassuming vegetable is at the center of a raging controversy—with the future of agricultural biotechnology in India hanging in the balance.

At a press conference here on 9 February, India's environment minister, Jairam Ramesh, announced a "moratorium" on commercial release of what would have been India's first genetically modified (GM) food crop: varieties of eggplant, called brinjal in India, equipped with a protein from the bacterium *Bacillus thuringiensis* (Bt) that's toxic to insect pests.

The moratorium overturns a regulatory panel's decision last autumn to clear Bt brinjal for commercial planting. But with public sentiments running strongly against Bt brinjal, Ramesh said, "it is my duty to adopt a cautious, precautionary, and principle-based approach." The moratorium will stay in place, he said, until studies establish "the safety of the product from the point of view of its long-term impact on human health and [the] environment."

Biotech boosters are reeling. "It could be a major setback for agricultural biotechnology in India," says Maharaj Kishan Bhan, an immunologist and secretary of the department of biotechnology in New Delhi. But some prominent voices had been preaching caution. "Who will have access to the technology? Who will be responsible if something goes wrong? We must address public concerns before making a decision," says agricultural scientist M. S. Swaminathan, chair of the M. S. Swaminathan Research Foundation in Chennai. Others cite possible toxicity of the Bt protein and the potential that it could contaminate non-GM brinjal. Ramesh has taken a "courageous stand," says molecular biologist Pushpa M. Bhargava, former director of the Centre for Cellular & Molecular Biology in Hyderabad, who calls the moratorium "fair and good."

India is the center of diversity for brinjal, with more than 2500 native varieties. It is also the second-largest producer after China—but yields have been hit hard by an insect called the fruit and shoot borer. The pest has caused



Turning point. Jairam Ramesh, India's environment minister, intervened in plans to release a genetically modified food crop, brinjal. His decision could have a big impact on biotechnology.



losses of up to 70% of commercial plantings, according to the International Service for the Acquisition of Agri-biotech Applications in New Delhi, a GM food advocacy organization. The group claims that farmers must apply pesticides to brinjal up to 40 times over the 120 days from sowing to harvest, and that pesticide use could be reduced with Bt plants.

Work on Bt brinjal in India began in 2000, when Maharashtra Hybrid Seeds Company Ltd. (MAHYCO) in Mumbai inserted the Bt *cryIAC* gene into eight hybrid brinjal varieties. (The Bt technology was licensed from Monsanto, which owns a minority stake in MAHYCO.) In compliance with Indian regulations, MAHYCO carried out toxicology and allergenicity studies, as well as field trials at 59 locations; of these, 42 were done independently in India's agriculture research system. An assessment of whether pollen from GM brinjal would contaminate native crops found "limited outcrossing" with Bt pollen traveling a maximum of 30 meters.

Last October, India's top biotechnology regulatory body, the environment ministry's Genetic Engineering Approval Committee (GEAC), concluded that "Bt brinjal is safe for environmental release" but deferred a final decision due to the "major policy implications." Ramesh withheld approval and announced a series of seven public hearings that wrapped up last week in Bangalore. The often-tumultuous hearings strengthened the hand of opponents: Even before Ramesh's decision, several Indian states announced that they would attempt to ban commercial

planting of Bt brinjal. (On 9 February, he took a swipe at GEAC, announcing that he would rename it the Genetic Engineering Appraisals Committee.)

Some influential scientists are thrilled by Ramesh's call for additional tests. "The safety assessment is not complete," argues Bhargava, a special observer to GEAC appointed by India's Supreme Court. He says MAHYCO should undertake another 30 tests, including chronic toxicity studies and a comparison of all proteins in Bt brinjal and a representative native variety—tests that he claims could take 22 years to complete. A 9 February statement from MAHYCO said the company "respects" Ramesh's decision. "We have no hesitation in doing more tests," Usha Barwale Zehr, a genetic engineer and MAHYCO's chief technology officer, told *Science*. "But it certainly can't be unending, and the new tests ... need to have a scientific value."

Supporters of GM crops take comfort in the fact that the moratorium applies only to eight Bt brinjal varieties and that Prime Minister Manmohan Singh's government has not rejected all GM technology. Indeed, brinjal would not have been the first GM crop in India: In 2002, the government approved Bt cotton, which is now cultivated on more than 9 million hectares in India. Last month, Singh declared that "we should pursue all possible leads that biotechnology provides that might increase our food security." But for now the country's first GM food has proved too hard to swallow. **—PALLAVA BAGLA**
With reporting by Richard Stone.

ScienceNOW.org

From *Science's*
Online Daily News Site

Better to React Than to Act

Have you ever noticed that the first cowboy to draw his gun in a Hollywood Western is invariably the one to get shot? Nobel Prize-winning physicist Niels Bohr did, once arranging mock duels to test the validity of this cinematic curiosity. Following Bohr's example, researchers have now confirmed that people move faster if they are reacting to another person's movements than if they are taking the lead themselves. The findings may one day inspire new therapies for patients with brain damage, the team speculates. <http://bit.ly/actreact>

Australia, Antarctica Linked by Climate

Researchers have found an intriguing climate link between southwestern Australia and eastern Antarctica. When the former suffers a drought, the latter is battered with snow. Even more provocative: Human activity seems to be driving the connection. <http://bit.ly/climatelink>



Can Thin Mountain Air Make You Slim?

Looking for a new weight loss plan? Try living on top of a mountain. Twenty obese men spent a week near the top of Germany's highest peak and saw their metabolism speed up, their appetite diminish, and more pounds melt off than they likely would have had they stayed at home, a new study reports. However, the study lacked a control group, so firm conclusions are tough to draw, other researchers say. <http://bit.ly/mountainair>

How Cancer Wreaks Havoc on Bone

An insulin-like hormone speeds the destruction of bone caused by malignant tumors, a team of clinical pathologists has found. If confirmed, the results could eventually point to drugs for slowing or stopping the damage to bones caused by cancers. <http://bit.ly/bonecancer>

Read the full postings, comments, and more on scienconow.sciencemag.org.

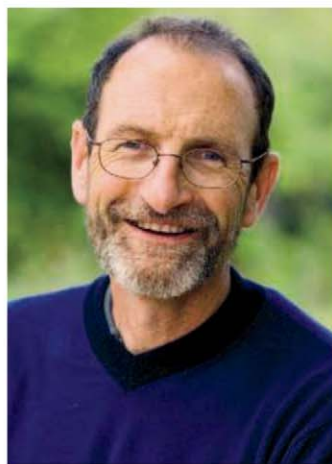
NEWSMAKER INTERVIEW

IPCC Seeks 'Broader Community Engagement' to Correct Errors

Amid mounting attacks on the Intergovernmental Panel on Climate Change (IPCC), a small number of its volunteer leadership has tried to respond to the horde of bloggers and reporters as well as explain themselves to colleagues. Prominent among them has been Christopher Field of the Carnegie Institution for Science in Palo Alto, California. For the 2014 edition he is co-chair of the report by Working Group 2, which will examine the impacts of a changing climate.

A field ecologist who made his name examining the interrelationship of nitrogen, plants, and atmospheric carbon, the outdoorsy Field has become increasingly comfortable in a suit and tie. Be it in congressional testimony or addressing delegates at the Copenhagen talks last December, Field delivers his scientific message in a deliberate and understated tone, in sharp contrast to Rajendra Pachauri, IPCC's volatile chair. "He's one of the most unflappable people I've been around, and I think you need someone like that to steady the ship when it's rocking," says biogeochemist Benjamin Houlton of the University of California, Davis.

Field has spent the past few months



Climate control. Chris Field is working to help IPCC improve its performance.

responding to critics of what IPCC has done and also discussing where it is headed. He helped write a rare public acknowledgment of error in a statement in the 2007 report saying that Himalayan glaciers were "very likely" to melt away by 2035. Despite the criticism of the panel, he says he's had early success recruiting authors for the next report, whose teams should be finalized by this summer. Here are excerpts from a 5 February phone interview with *Science*.

—ELI KINTISCH

Q: You've said that the error about melting glaciers was a case of not following IPCC's own procedures. But you also say that the community should have pointed out the error during review opportunities.

C.F.: It's clear there really wasn't a body of evidence required to assign that "very likely" term. So the procedures really weren't followed. But I think it's also clear that the procedures are set up so that had there been more review comments, had the level of expertise on the topic been recruited as a [chapter contributing author], there were a bunch of places where the error could've been avoided with a broader community engagement.

U.S. SCIENCE POLICY

Bement to Leave NSF Before Term Ends

The last major science holdover in the Obama Administration is stepping down ahead of schedule this spring, leaving the president free to appoint a new director for the \$7 billion National Science Foundation (NSF).

Arden Bement announced last week that he will be leaving NSF on 1 June to lead a new public policy institute at Purdue University, where he's been on leave since 2001. A nuclear engineer, Bement led the National Institute of Standards and Technology before being appointed acting NSF director

in 2004 following the resignation of Rita Colwell. Several months later, President George W. Bush nominated him for a full, 6-year term that expires in November 2010.

A nuclear engineer who came to Purdue in 1992 after a long career in industry, Bement gave the incoming president little reason to replace him at the helm of an agency that has traditionally enjoyed bipartisan support. The Obama Administration has pledged to continue a 2006 promise by Bush to double NSF's budget, although it extended

Q: Critics say that IPCC should develop a means for formal corrections, like those used by scientific journals.

C.F.: The reason that is tough is because the IPCC relies so heavily on this multiphase review and approval mechanism. ... It's hard for me to figure out what might be a process that would sustain the credibility that should be associated with the IPCC process. Ideally, a correction would go back through an IPCC-type process. But that would take as long as producing the next report. ... I must admit I don't really have a mature strategy for how we deal with [substantive] errors. One possibility might be if the IPCC were to write a "Special Report" to update each assessment. We have a well-established mechanism to do these.

Q: Apart from the issue of the Himalayan glaciers, there have been recent criticisms of how the 2007 report dealt with disaster losses and the Amazonian rainforest, among a series of others that have cropped up.

C.F.: [They] don't have any real substance. The report is standing up incredibly well to a blizzard of attacks.

Q: Should the IPCC authors who fail to catch errors face official consequences?

C.F.: Every scientist does their best with each paper or work that comes out of their group. ... Having your work criticized in a public way is a difficult [enough] situation for a scientist. ... With a mistake like the Himalayan glaciers one, there's plenty of blame to go around.

Q: Are you happy with how the IPCC leadership has responded to an almost unprecedented amount of public criticism?

C.F.: I think it's fair to say that nobody was expecting this, and nobody was prepared for it, including me. It's taking a long time for us

to get our act together in terms of communicating who we are and what we do. ... It would have been nice if we had a rapid-response way of checking out any challenges that arise, and having time to respond in kind. I think we're also struggling to explain what the IPCC is, its procedures.

Q: Member governments pay for IPCC. What are they saying?

C.F.: We haven't had a systematic set of responses from governments. I think it's fair to say there have been some expressions of concern.

Q: Some have said that Pachauri has been too defensive or activist in his responses to the criticism. Do you think he's done a good job?

C.F.: I think he's tried to do the best job he can do.

Q: There is a growing community of critics and bloggers who are publishing information on climate change outside of the established community. Is there a place for this science along with the peer-reviewed literature?

C.F.: In the long run, we should take advantage of the benefits of both and not suffer the weaknesses of either.

Q: You were a co-leader of the chapter on North America in the 2007 report. Why did you seek to expand the role for the next report?

C.F.: I feel a responsibility as a member of the scientific community. ... While there have been a number of aspects of the last few months that have been frustrating, I'm really proud to be a member of an activity that has in the past provided so much value to the public on the issue of climate change, and I'm confident I can continue to do that.

the time frame from 7 to 10 years. Last week, President Barack Obama requested an 8% increase for NSF in his 2011 budget despite proposing a freeze on overall domestic discretionary spending.

At an age when most people are already retired, Bement says, "I feel like 77 going on 45." His return to West Lafayette, Indiana, will certainly be a homecoming for him. The Global Policy Research Institute that he will lead is part of a strategic plan hatched by Purdue's president, France Córdova, who is a member of the National Science Board, NSF's



Arden Bement.

oversight body. And Richard Buckius, Purdue's vice president for research, served as head of NSF's engineering directorate under Bement before going to Purdue in 2008.

Bement's immediate successor is expected to be Cora Marrett, who came to NSF in 2007 to head its education directorate and has served for the past year as acting deputy director. But Marrett may also be a short-termer unless she is chosen for the top job, giving the Obama Administration a chance to fill both positions.

—JEFFREY MERVIS

ScienceInsider

From the Science Policy Blog



A big winner in the recently proposed 2011 federal budget was the **Advanced Research Projects Agency-Energy**, which received a \$300 million boost—\$75 million more than the entire Office of Science, the Energy Department's basic research arm. Energy Secretary Steven Chu explained that the boost was needed to provide a "quick hit" on advanced energy concepts. <http://bit.ly/bWcaOM>

The National Oceanic and Atmospheric Administration wants to fly **DSCOVR**, a controversial satellite first proposed by former Vice President Al Gore. The craft would sit 1.6 million kilometers from Earth, between our planet and the sun, and gather data on space weather. Earth scientists hoping to use the satellite for climate research are awaiting a NASA decision on whether to do so. <http://bit.ly/a7RXjS>

A patent on creating induced pluripotent stem cells was granted to two Boston-area researchers, leading some to wonder if the rush for intellectual-property rights will slow clinical development of this promising technology. <http://bit.ly/aenzs2>

A court at The Hague last week dealt a blow to the Dutch government's controversial policy to **exclude Iranian-born students and scientists** from master's degrees involving nuclear technology and from nuclear research facilities in the Netherlands, calling the ban overly broad and a violation of an international civil rights treaty. <http://bit.ly/cbn2dL>

The Obama Administration announced a new **strategy** for preventing an invasive species, the Asian carp, from entering the Great Lakes, where it could threaten a sportfishing industry worth \$7 billion. The plan also includes money for research on how to battle the fish. However, the move appears unlikely to end a feud between midwestern states over what to do about the carp. <http://bit.ly/bAbbO5>

For the full postings and more, go to blogs.sciencemag.org/scienceinsider.

PSYCHIATRY

Proposed Revisions to Psychiatry's Canon Unveiled

This was a momentous week for psychiatry. After more than a decade of labor, the American Psychiatric Association (APA) has released draft proposals for the upcoming fifth version of the *Diagnostic and Statistical Manual of Mental Disorders (DSM-V)*, the most influential book in psychiatry. By classifying mental disorders and giving them names, *DSM* not only influences how doctors diagnose and treat their patients, but it also sways how insurance companies decide which conditions to cover, how pharmaceutical companies design clinical trials, and how funding agencies decide which research to fund. Making changes to such a widely used document was bound to be controversial, and it has been. "It's sort of like repairing an airplane while it's still flying," says psychiatrist Steven Hyman, provost of Harvard University and a member of the committee leading the *DSM-V* revisions.

Among a number of new proposals that seem likely to cause a stir are a diagnosis of "prepsychotic risk syndrome" applicable to young people and a redefinition of autism spectrum disorders that would eliminate Asperger's syndrome, which many consider a

mild form of autism. (For highlights of the proposed changes, see *DSM-V* at a Glance; to view the proposals, go to www.dsm5.org.) After gathering comments on the draft criteria and conducting field trials to test them, APA plans to publish the finished edition in 2013.

The researchers and clinicians working on *DSM-V* had several ambitious goals, including using new findings from neuroscience and genetics to shape diagnoses, minimizing vast diagnostic dead zones of abnormal behavior that fall in the cracks of current criteria, and introducing the idea of "dimensions" to reflect varying degrees of symptom severity and the overlap among disorders.

The current fourth edition of *DSM* was originally published in 1994 and revised in 2000. By that time, some experts already envisaged a far grander revision based on rapidly advancing scientific knowledge: genetic findings that reveal commonalities between bipolar illness and schizophrenia, for example, and brain-imaging studies that differentiate the neural circuitry involved in compulsions and addictions (*Science*, 31 October 2003, p. 808).



When the first planning conference for *DSM-V* was held in 1999, participants had high hopes for undergirding many diagnoses with specific biological indicators such as brain scans or genetic tests. In the end, the revision process, led by psychiatrists David Kupfer of the University of Pittsburgh in Pennsylvania and the APA's Darrel Regier, found no such markers that could serve as reliable diagnostic guides. Instead, the work groups focusing on specific areas were asked to consider biological factors among 11 indicators of diagnostic validity. (Others include environmental risk factors, personality traits, and treatment responses.)

The *DSM-V* work groups have attempted to close some of the gaps between diagnoses. Since the 1970s, *DSM* editions have sought to put diagnoses on a more empirical basis by describing and categorizing symptoms without recourse to theory or assumptions about causes. But as a result, many

types of abnormal behavior didn't earn any clear diagnosis and were designated "not otherwise specified" (NOS). This category is widely used by clinicians, says Regier—and "when you have 40% of academic inpatient facilities discharging people with NOS diagnoses, you know you have a problem."

Another problem with *DSM-IV* was its all-or-nothing approach: Check five of nine symptoms for depression and the patient has

DSM-V at a Glance

Psychotic Disorders

Old subtypes for schizophrenia will be discarded. Diagnosis will be made based on common symptoms such as hallucinations and thought disorder, as well as their duration and severity.

Newly proposed is "psychosis risk syndrome" for people showing warning signs such as delusions, hallucinations, or disorganized speech and experiencing distress. Critics say this could stigmatize many young people. Defenders say early identification could help them.

Mood Disorders

DSM-IV lists nine symptoms on which to base diagnosis of depression. The proposed one emphasizes three basic dimensions: depression with anxiety, with substance abuse, and with suicidality. A new diagnosis of "mixed anxiety depression" is proposed. The threshold for bipolar diagnosis is lowered slightly, to accommodate depression with only one or two

episodes of mania. This change recognizes the fact that some antidepressants can trigger a manic episode in the vulnerable.

Anxiety Disorders

The main change is the expansion of obsessive-compulsive disorder (OCD) spectrum, which now pulls in disorders from far-flung parts of *DSM-IV*. These include Tourette syndrome, body dysmorphic disorder (obsession with changing a normal body part), and trichotillomania (hair-pulling). "Hoarding disorder" has also been added to the spectrum. There is still debate over whether OCD should have a designation separate from anxiety disorders.

Personality Disorders

The old *DSM* laundry list of 12 personality disorders will be trimmed to five: borderline, schizotypal, avoidant, obsessive-compulsive, and antisocial/psychopathic. ("Psychopathic," eschewed in earlier *DSMs*, is now back.) The other diagnoses will be superseded by a "mix

and match" menu of symptoms that reflect two types of core pathologies: disturbances related to self-concept, and those related to interpersonal functioning such as cooperativeness and empathy.

Addiction and Related Disorders

Vocabulary is being overhauled. "Dependence" (which implies physical and not necessarily psychological dependence) is out. "Abuse" is also out as unsupported scientifically. Instead, varying degrees of "use disorder," as in "alcohol use disorder," are proposed.

"Gambling disorder" has achieved the status of addiction, based on behavioral and biological similarities to substance addiction. "Internet addiction" is under consideration but hasn't yet made the grade.

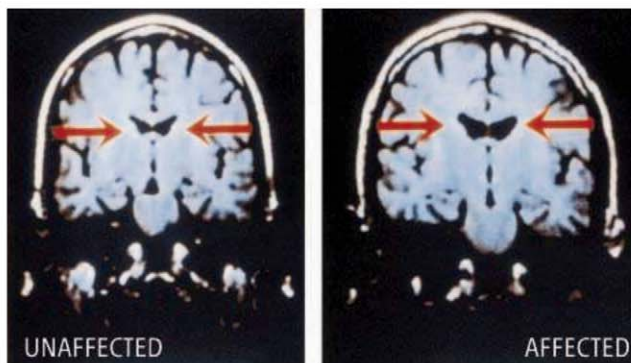
Eating Disorders

New addition is "binge eating," which has been moved from the *DSM* Appendix to become a full-fledged disorder.

it; check four of nine and he doesn't. Kupfer says a central innovation in *DSM-V* is the concept of "dimensions" intended to enable clinicians to gauge the severity of a patient's condition by rating factors such as subjective distress and degree of impairment in addition to symptoms. Dimensions also allow disorders to be deconstructed into components that can be addressed separately, such as the depression that accompanies many disorders. This approach acknowledges that "pure" disorders are rare, and comorbidity is the norm, Kupfer says.

The revision process has drawn criticism from various quarters. Psychiatrists Robert Spitzer, guiding force behind *DSM-III*, and Allen Frances, who headed the *DSM-IV*, have complained that too much of the work has been done behind closed doors. Others have voiced concerns about having researchers with a history of financial ties to pharmaceutical companies (as do a majority of those in the work groups) write the manual.

Work group members have had to confront both political pressure and scientific dilemmas. The transgender community has been worried about the stigmatizing effect of the current term "gender identity disorder," because they believe they are just normal people caught in the wrong-sex body. Suggestions for a "pre-psychotic syndrome" have some worried that many normal adolescents will be wrongly treated with powerful antipsychotic drugs.



Twin mystery. Scans of identical twins, one with schizophrenia (right), reveal differences in the brain, but they're not reliable enough for a diagnosis.

Similarly, there's concern that "hypersexuality," championed by some as justified by the literature, is a diagnosis with no clear limits.

Frances and other critics question the entire *DSM-V* enterprise, arguing that a major revision should have been put off until there are more hard data on biological causes of mental disorders. Adding "dimensions" is of dubious benefit, says psychiatrist Michael First, editor of the *DSM-IV*. Dimensions are what the working groups are "left with after etiology fell through."

"I've always been uncomfortable with the idea of producing one large book that changes everything," adds psychiatrist Jane Costello of Duke University in Durham, North Carolina, who resigned last year from the work group on child and adolescent disorders. Costello says she'd rather see a Web site set up where changes to the diagnostic criteria could be made when—and only

when—sufficient scientific evidence supports it. Kupfer says that's just what the framers of the *DSM-V* intend to happen in the future.

Many questions remain, but time and money are limited. APA has pledged \$25 million for the entire *DSM-V* project, a woefully inadequate sum, according to some critics. Clinicians and researchers will now have 3 months to weigh in on the proposed changes. Field trials, involving perhaps 5000 patients, are scheduled to start in July, Regier says. These will test major changes and new diag-

nososes in a variety of ways, such as comparing *DSM-IV* and *DSM-V* diagnoses on the same patients, or ascertaining whether two practitioners agree on a diagnosis for the same patient. New scales will also be tested. The work group on depression has devised a six-factor suicide-prediction scale, for example. Psychiatrist Jan Fawcett of the University of New Mexico, Santa Fe, says the trials should help determine "how many dimensions we can get away with" in view of the fact that clinicians average about 15 minutes per patient. Trials should also reveal whether critics' fears of "false epidemics" of mental illness caused by more expansive diagnostic criteria are justified.

In the coming weeks, *Science* will explore several of the new proposals—and researchers' and clinicians' reactions to them—in more detail. First up, in next week's issue, a closer look at addictions.

—GREG MILLER AND CONSTANCE HOLDEN

Sexual and Gender Identity Disorders

"Gender identity disorder" has been retained despite pressure from transsexual advocates. Several new diagnoses, including "sexual interest/arousal disorder in women," are proposed. The most controversial is a proposal for "hypersexual disorder," involving recurrent and distressing sexual "fantasies, urges and behavior."

ADHD and Disruptive Behaviors

Changes to attention disorder diagnoses are still under consideration. The group proposes a new subtype of conduct disorder that includes callous, unemotional traits (such as lack of guilt or remorse), citing recent evidence that this subset of children and adolescents may be more prone to chronic violent behavior and require different types of treatment.

Childhood and Adolescent Disorders

Additions include specific criteria for diagnosing post-traumatic stress disorder in preschool children and "temper dysregulation disorder with

dysphoria," characterized by severe temper outbursts alternating with negative mood states. Children with this problem are often diagnosed with juvenile bipolar disorder under *DSM-IV*.

Neurocognitive Disorders

This new category would subsume various *DSM-IV* diagnoses, dividing them into major and minor disorders. Major neurocognitive disorders (such as various forms of dementia) involve a decline that interferes with independent living. Minor disorders would include mild cognitive impairment (MCI), a suite of memory and other problems considered a possible prelude to Alzheimer's. Elevating MCI to a formal diagnosis could facilitate clinical trials aimed at preventing Alzheimer's.

Neurodevelopmental Disorders

Several *DSM-IV* diagnoses would be consolidated into a single, broader diagnosis of "autism spectrum disorders." These include Asperger's syndrome, a high-functioning form

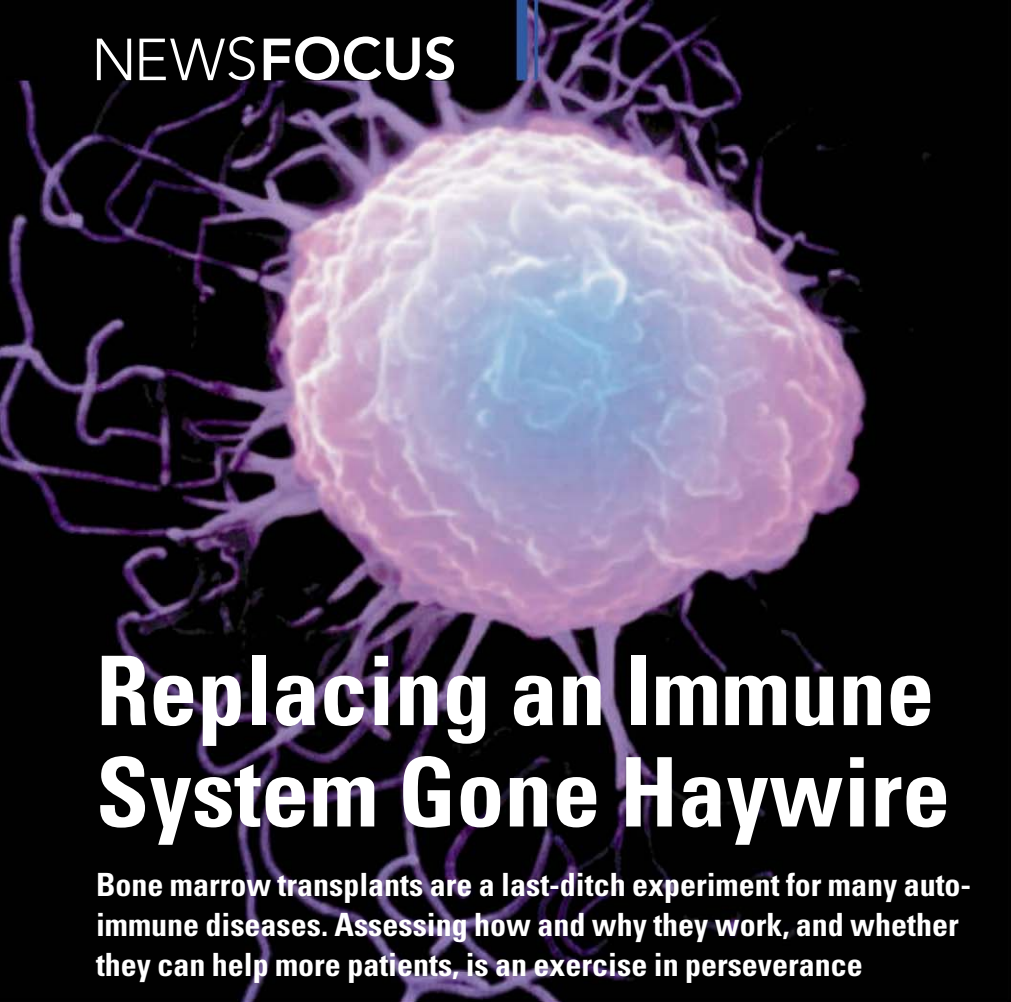
of autism. The group says there is no scientific justification for the term, but the change has been strenuously resisted by Asperger's advocates. Also, the term "mental retardation" would be replaced with "intellectual disabilities."

Sleep-Wake Disorders

DSM-IV distinguishes "primary insomnia" from insomnia caused by other conditions. These would be merged into a single diagnosis in *DSM-V*, with clinicians asked to note accompanying dimensions such as depression or heart disease. "Restless leg syndrome" would be elevated to a formal diagnosis.

Somatic Distress Disorders

Several diagnoses that deal with bodily complaints would be folded into a new umbrella diagnosis of "complex somatic symptom disorder" on the grounds that *DSM-IV* diagnoses such as somatization disorder and hypochondriasis have common features such as chronic physical complaints and distorted perceptions of symptoms.



Replacing an Immune System Gone Haywire

Bone marrow transplants are a last-ditch experiment for many autoimmune diseases. Assessing how and why they work, and whether they can help more patients, is an exercise in perseverance

In the fall of 1996, more than 200 immunologists and oncologists gathered in Basel, Switzerland, to discuss a drastic, life-threatening strategy to beat back autoimmune disease: Destroy a patient's immune system with a blitz of chemotherapy and radiation before providing them a bone marrow transplant. Then watch and wait and hope the immune system is reborn, pristine and free of disease.

Bone marrow transplants, now called hematopoietic stem cell transplants, had been part of oncology's arsenal for many years to rid patients of blood cancers—and many patients have died from the intensity of the transplant or its aftermath. But that September more than 13 years ago, there was optimism, from animal studies and a handful of anecdotes in humans, that gentler transplants were possible and that they might reset a malfunctioning immune system as no other treatment could. The Basel group set out to test their hunch, launching a number of small clinical trials.

In medicine, mainstream treatments often start as the therapy of last resort: toxic, risky, desperate strategies to save the sickest patients. Time refines them; science clarifies who will benefit and who won't. To date, roughly 1500 adults and children worldwide

have received stem cell transplants for a host of autoimmune diseases, including multiple sclerosis (MS), scleroderma, lupus, diabetes, and juvenile arthritis. Nearly all have been carefully tracked and monitored, with many giving blood and other tissue so that scientists can parse the evolution of their new immune system over months and years.

The results have been mixed, but there are startling success stories: About one-third of participants—many debilitated by their disease, in wheelchairs, or facing imminent death—go into remission and no longer need medication long-term, something that can't be achieved with existing treatments. Another third benefit, but only for a year or two, before relapsing. And a third don't respond at all, with about 1% to 5% dying from the treatment.

Scientists can't yet explain why some do so well following transplant and others don't, partly because they don't understand how, exactly, the transplants are rewiring a faulty immune system. And they worry that even as the field matures and the number of trials expands, assessing how well transplants really work is growing ever more difficult. Roadblocks include paltry funding—the trials lack commercial support because they're not testing new drugs—and difficulty finding

Back in charge. There are hints that regulatory T cells tame the immune system after a transplant.

patients, because rheumatologists and neurologists are skeptical of transplants, and new, promising, and generally safer biologic therapies are competing for patients' attention.

There's also growing evidence that stem cell transplants work best in healthier people whose disease hasn't damaged major organs. But for the most part, those aren't the patients receiving transplants: The toxicity of the treatment, uncertainty over how best to coax it to work, and tight restrictions from regulatory agencies over whom to transplant mean that many studies are restricted to the sickest of the sick—and that the therapy risks performing below its full potential.

Pressing the reset button

Physicians came to transplantation from different starting points. For Keith Sullivan, an oncologist and transplant physician at Duke University in Durham, North Carolina, success in a disease outside his area drew him to autoimmune conditions. In the 1990s, he and his colleagues found that young adults with sickle cell disease, which causes excruciating pain and strokes, responded remarkably well to stem cell transplants. "We said, 'Okay, ... you can put a new blood-forming system in a patient with sickle cell disease and essentially cure' " that person, something not possible with existing treatments. So why not try "putting a new immune system in a patient with autoimmune disease?"

In stem cell transplants for cancer, patients are generally bombarded with near-lethal doses of chemotherapy and often radiation, which wipe out blood-forming cells in the marrow—along with any lingering malignant cells—to make room for healthy cells infused from a donor. Over time the donor cells proliferate, spawning a new blood system of T cells, B cells, and other immune components.

Most cancer patients undergoing transplants will die from their disease without one. Autoimmune diseases are less often fatal. Because of that, physicians focused on safer autologous transplants, which use cells from the patient, rather than allogeneic ones, in which cells are drawn from a donor, such as a sibling. In the late 1990s, when transplants for autoimmune diseases began in earnest, 3% to 5% of patients died from autologous transplants; 15% to 35% died from allogeneic ones.

Transplant physicians worried, however, whether they would be trading safety for effectiveness. If their patients' cells were pre-

disposed to attack their own tissue, wouldn't the disease come back after reinfusing them? "That's what we kind of thought going into this," says Sullivan.

He focused on one of the most vicious autoimmune diseases, a severe form of scleroderma called systemic sclerosis, for which there are few treatments and high rates of mortality. Like other transplant physicians working on autoimmune conditions, Sullivan also dialed down the toxicity of the treatment pretransplant because he didn't need to destroy cancer cells, too. First, he collected blood from his patients and singled out CD34 progenitor cells—primitive blood cells that differentiate into more mature blood and immune players. These are the cells his patients would receive in the transplant.

Meanwhile, other physicians were experimenting as well. Paolo Muraro, a neuroimmunologist now at Imperial College London, was working at the U.S. National Institutes of Health in Bethesda, Maryland, from 2001 to 2005, studying blood cells from patients with MS who had received stem cell transplants to treat their MS. "The first question we asked: Is there the so-called immune resetting" after transplant? "Does it actually take place?"

Studying these cells, gathered over time, Muraro discerned a large number of T cells

A SAMPLING OF TRANSPLANT TRIALS

Disease	Number Enrolled	Enrollment Goal	Principal Investigator	Status
Multiple sclerosis	Not available	155	Richard Burt, U.S.	Ongoing
Multiple sclerosis	28	25	Richard Nash, U.S.	Ongoing
Multiple sclerosis	21	200*	Gian Luigi Mancardi, Italy	Closed due to lack of participants
Scleroderma	156	150	Jaap Van Laar, U.K.	Transplants complete, follow-up continues
Scleroderma	Over 170**	100	Keith Sullivan, U.S.	Ongoing
Type 1 diabetes	23	12	Júlio Voltarelli, Brazil	Transplants complete, follow-up continues
Crohn's disease	20 [approx.]	48	Christopher Hawkey, U.K.	Ongoing

*Enrollment goal later scaled back to 30 and trial redesigned

**More patients are enrolled than can participate, because insurance often declines to pay for transplants. 60 have been randomized so far.

that had recently filtered out of the thymus—an indicator that they were newly formed. "It was not 100% renewal," he says; some cells that were present pretransplant remained. But enough young T cells were flourishing that Muraro concluded that a new immune system had seeded. He published the work in 2005 in *The Journal of Experimental Medicine*.

The lab findings matched what physicians were seeing in some patients. Sullivan's fear of a disease resurgence after a transplant did come true for certain individuals, but others stayed in remission for years. He attributes that to the particular set of circumstances that launched an autoimmune attack initially, some combination of environmental triggers, such as a viral infection, and unlucky genetics. Because the new immune system regenerates later in time, the environmental factors that originally triggered autoimmune attacks may be absent. "That may trump the fact that you have genetic predisposition," Sullivan says.

More recently, a number of studies have dug deeper, probing how the transplants are altering immunity. Last year, a German group described findings from five people with lupus who had been in remission for as long as 8 years since their transplants. All five had lost pathogenic antibodies linked to lupus, and the number of B cells in their blood had normalized. Other researchers are finding hints that in various diseases, regulatory T cells, which keep the immune system from acting out, flourish post-transplant.

These are just pieces of a larger puzzle, and it has many gaping holes. "There's a huge black box here: Why is this working?" asks Ann Woolfrey, a pediatric hematologist-oncologist at the Fred Hutchinson Cancer Research Center in Seattle, Washington. It's not clear which cells must be destroyed

prior to transplant. Nor is it known which ones keep disease at bay afterward.

Slow ahead

In some, however, the transplants work wonders. In 2006, researchers reported that 50% with lupus remained in remission, along with about 30% who had either MS or scleroderma. A team of Europeans last year looked back over 12 years and 900 transplants and found that 59 patients had died from transplant-related complications, and about 40% had experienced no disease progression.

But as hopeful as most of these numbers are, nearly everyone agrees that stem cell transplants will remain forever experimental unless they compare favorably to other treatments, particularly in their ability to induce lasting remission. Although most physicians agree that patients should try safer therapies first before resorting to a risky stem cell transplant, even the best biologic therapies hitting the market won't work for everyone—and when they do help, they must often be taken for life. Randomized trials to match transplants against standard therapy mean juggling stringent regulatory requirements, a constant need for funding, and sluggish patient recruitment. "It takes time and endurance" to pull this off, says Alan Tyndall, a rheumatologist at the University of Basel, and, with Basel transplant physician Alois Gratwohl, a pioneer in the field. "It's exhausting."

One of the biggest challenges has been finding patients. A European trial for MS closed in December after recruiting just 21 people out of the once-hoped-for 200. In pediatrics, Woolfrey and her colleague Carol Wallace, at Seattle Children's Hospital, have sought patients for more than 5 years for a trial in pediatric autoimmune disease and transplanted only four, all with juvenile arthritis. Another study of pediatric autoimmune disease, led by Mitchell Cairo, a pediatric hematologist-oncologist at Columbia University, shut down several years ago. "We couldn't get rheumatologists to [refer] patients," says Cairo, who performed just two transplants for the study before giving up.

The problem, physicians agree, is that transplant experts, accustomed to treating cancer patients in dire straits, eye risk through a fundamentally different prism than do the neurologists, rheumatologists, and other spe-



Weighing the alternatives. The option of new biologic therapies, which this little girl is receiving for her juvenile arthritis, make trial recruitment difficult.

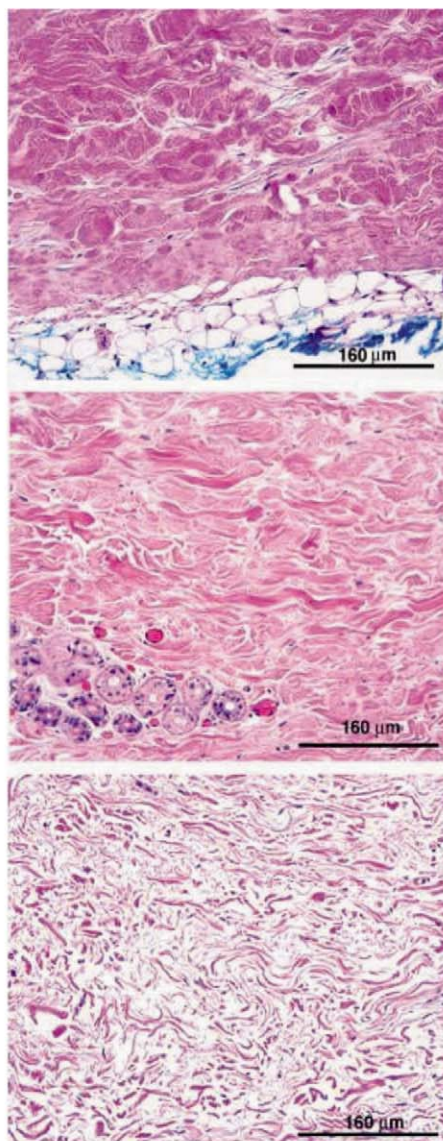
cialists who see autoimmune patients day in and day out. “From a transplant perspective, 5% mortality [from the treatment] is great,” says Camillo Ricordi, scientific director at the Diabetes Research Institute at the University of Miami in Florida. “In a diabetes treatment, 1% mortality will be unacceptable.”

Death rates from the transplants have dropped in the past 10 years, although they vary depending on the approach. Some physicians are experimenting with riskier allogeneic transplants in small trials, collecting cells from donors that they believe make a cure more likely. Others are moving in the opposite direction, jettisoning radiation and lightening the chemotherapy load as much as possible.

Physicians are also walking a tightrope in identifying which patients to transplant. “Transplantation is what you call a one-shot treatment,” which makes picking the right patients critical, says Riccardo Saccardi, who performs bone marrow transplants at the Careggi Hospital in Florence, Italy, and who also chairs the working party on autoimmune diseases of the European Group for Blood and Marrow Transplantation. Early trials in those with advanced MS generally failed to help; trials in scleroderma on people with severe lung disease had high mortality.

In choosing patients for trials, many physicians are torn between instinct and reality. Their gut tells them that the therapy is most likely to help those early in disease, who don’t yet have damage to their brain, their kidneys, or their lungs. But the risks of transplant, and uncertainty around whose disease will progress without it, makes transplanting such patients ethically questionable.

Some have forged ahead regardless. In January 2004, clinical immunologist Júlio Voltarelli of the University of São Paulo in Brazil and Richard Burt, who oversees immunotherapy for autoimmune disease at Northwestern University in Chicago, Illinois, began transplanting teenagers and young adults with type 1 diabetes, after spending more than 2 years seeking, and achieving, approval from an ethics board in Brazil. Their rationale: Diabetes destroys insulin-producing cells in the pancreas soon after diagnosis, and the window to act is a narrow one. Voltarelli has done 24 transplants and published findings from most of them in 2007 and 2009 in *The Journal of the American Medical Association*. “We can induce remission in almost all patients,” he says, although about half later relapsed and resumed insulin therapy



Calm after the storm. A scleroderma patient suffered hardening of the skin (*top*), with collagen deposits in dense pink. One year after transplant (*middle*), skin was improving, and 5 years later, it was back to normal (*bottom*).

The diabetes study startled the field. “There was a lot of concern, taking these otherwise healthy individuals and giving them high-dose chemotherapy,” says Richard Nash, a transplant physician at the Fred Hutchinson. In diabetes, many young patients don’t develop major complications from the disease, such as kidney failure, for decades. Although none of the Brazilians died from the transplant, several suffered serious side effects, such as severe pneumonia and low sperm count that could affect fertility.

Still, the work has intrigued those who treat diabetes. “They show that you can stop the clock of autoimmunity,” says Ricordi,

who is interested in examining the treatment himself.

Burt argues that the chemotherapy given was relatively mild compared with that used in other studies—and that “there is no need” for more toxic regimens that some transplant experts are promoting. Others dispute that, saying that killing more cells up front in the patient may help a new immune system take root. Two ongoing trials in scleroderma should go a long way toward answering this question. In Europe, researchers have randomized 156 patients with the disease, with half receiving chemotherapy and then a transplant; in the United States, a similar trial takes a much more aggressive approach, by adding high-dose radiation. Both are at least 2 years away from reporting results.

That the scleroderma trials will even run their course is considered an enormous accomplishment. In the United States, insurance companies often decline to pay for the transplants, deeming them too experimental, thereby limiting trial enrollment; commercial funding is not an option because new drugs are not being tested. In Europe, government restrictions often control how many transplants can be performed at a given site. At University Medical Center Utrecht in the Netherlands, for example, national insurance companies will pay for about 35 stem cell transplants a year, says Nico Wulffraat, a pediatric rheumatologist at the hospital. Most of those go to cancer patients.

Clinical trials for new biologics also compete for the same participants, and that makes recruitment even harder, says Tyndall. Burt is running an MS trial and is recruiting in São Paulo and Prague, as well as Chicago and Calgary. Regulations around cell-therapy trials in the United States are so stringent as to virtually halt clinical research, many transplanters complain. “We’re blocking this with incredible rules and requirements before you even do a pilot trial,” says Ricordi. He is working with centers in China and Argentina on other types of cell transplants for diabetes to get around the roadblocks.

Tyndall hopes that the scleroderma trials will change the landscape. “If we can show with a disease like scleroderma, where there’s nothing else to offer, that it actually does put people into long-term remission,” then transplants might shift toward mainstream medicine. The therapy’s hazards are “pretty clear,” he says. The question is, “Which patients would justify that risk?”

—JENNIFER COUZIN-FRANKEL

NEWSMAKER INTERVIEW

Down-to-Earth Science Fiction

In his first novel, E. O. Wilson reaches into his ant drawer and his Alabama past to save an ecosystem

Harvard University biologist Edward O. Wilson has long attracted attention for his scientific studies of ants, his push to preserve biodiversity, and his controversial ideas on sociobiology. Now he's trying his hand at a different type of writing: fiction. His first—and, he says, last—novel, *Anthill*, combines a multigenerational saga based in rural southern Alabama, where Wilson grew up and still visits frequently, with an unusual novella about his favorite insect. In the novella, excerpted recently in *The New Yorker*, *Anthill*'s protagonist, Raphael Semmes Cody, captures the workings of an ant colony in vivid detail. Those ants are symbolic of one of the area's last remaining tracts of longleaf pine savanna. In the forthcoming novel, Cody, who explored this tract as a child, grows up to be a lawyer and a naturalist whose central goal becomes saving the land from developers. Wilson spoke with *Science* about tackling a new literary genre.

—ELIZABETH PENNISI

Q: How did this book come about?

E.O.W.: I always had in the back of my mind the possibility of writing a novel because of the challenge. It's a totally different way of thinking, creatively. It's harder than nonfiction because with nonfiction you can have the literature and the basic database in place and then piece your work together, coming back to it and letting it go for a while. When you are writing a novel, you have to create that world in your head and carry it around in your head.

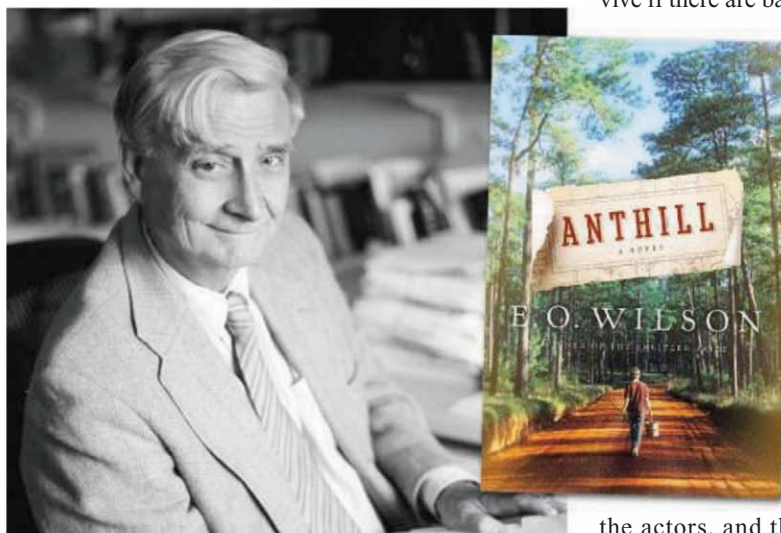
I created a fictitious county, northeast of Mobile, called Nokobee County. There [was] a natural environment that I wanted to be central to the novel, which is the longleaf pine savanna. It once covered 60% of the southeastern United States. When the South was recovering from the Civil War, the landowners cut down almost all original savanna to build wealth again. I made that [savanna] the focus of the conflict to be developed in the novel.

Q: Do you go back there a lot?

E.O.W.: I go back frequently. I think that the critical issue facing the people in the South, although I don't think they yet quite see it this way, is land, how they are going to treat land and their natural resources. They have been gobbling them up fast. One novel, *To Kill a Mockingbird*, had substantial impact on the self-image of the South. I am trying to develop a new self-image by writing this particular book.

Q: So how do you bring the ants in there?

E.O.W.: What are the animals that dominate the environment: Ants! I have used them to represent in some detail the Nokobee Tract and therefore the natural environment of



the South. Of course, that's not what most people think of as nature. They think of trees and bears and deer and snakes even, and so on.

I use [ants], because I know them intimately. They go through battles, through tournaments, through the death of the queen, and through the death of the entire colony. There are parallels with cycles of human civilization.

Q: How about the scientific accuracy? Did you take literary license with the *Anthill Chronicles* [the novella]?

E.O.W.: Some, but not enough to outrage my colleagues.

Q: How do you know it wasn't enough?

E.O.W.: Because Bert Hölldobler [Wilson's co-author on the recent book *The Superorganism*] is a purist in writing about scientific subjects, including ants, but Bert told me he thinks it's marvelous. I was concerned. I take license, there is no question about it. Everything about the ants in there is based on fact, except I have mental activities occurring, and I stretch it pretty far.

The chronicles will present to the reader for the first time ever what ants really do, what they experience, how they talk to one another, in pheromones, what happens through the life cycle of a colony, and how they conduct their wars. All of that is fact-based, and it's pretty close to the real thing.

Q: Are you worried about the critics in terms of having a novel that's out there and being evaluated as a literary piece?

E.O.W.: I realize I'm in another ballpark. But I've already gone back to science. I'll survive if there are bad reviews.

Q: Is Stephen Spielberg or Pixar vying for movie rights yet?

E.O.W.: It could be a movie. It would make a very good movie, I think. You decide. You read it.

Q: I immediately thought of *A Bug's Life* and *Antz*.

E.O.W.: Those movies have nothing to do with the real life of ants. They are about

the actors, and they are about what kids and adults think are funny about ants and other creatures. One good thing I hope will come from this book is that it will get the story straight and people will find out that ants are vastly more interesting than depicted in any movies that we have ever had or any television specials.

Q: Do you think fiction is harder to write than nonfiction or scientific literature?

E.O.W.: It certainly is for a scientist who spends all his time writing nonfiction. I had to completely retool my thinking: the way I created things in my mind and the way I found expressions for them. I have tried to create something really new. I think the scientists will really like it.

Racing Crash-Happy Cockroaches

No roach would ever make an Olympic gymnastics team. Although the American cockroach is lightning fast, traveling more than 1.5 meters per second and able to scale walls in the blink of an eye, it would win no awards for grace or flawless performance, says Kaushik Jayaram, a graduate student in biomechanics and robotics at the University of California, Berkeley (UCB). At the meeting, he described experiments with high-speed cameras that showed that these insects' locomotion is anything but perfect, a finding with implications for the design and control of tiny robots. "We see our results as a paradigm shift for understanding effective performance," says Jayaram's adviser, Robert Full. "A successful performance must include a greater emphasis on robustness, not necessarily the most elegant solution of motion without error or missteps."

Jayaram was trying to understand how the insects use their eyes and antennae to switch from running flat out on a horizontal surface to climbing a wall, a transition that looks smooth to watching humans. Working with fellow graduate student Jean-Michel Mongeau and undergraduate Brian McRae, Jayaram built an enclosed, 55-centimeter-long runway with a wall at one end. He startled the cockroaches and then filmed them as they raced down the runway and up the far wall. He tested



the insects under low, ambient, and no light conditions on surfaces that included sandpaper and soft foam. Then he played the video back at slow speeds.

Often, "instead of using their head and associated sensors for neural control, they used their head like a car bumper," he reported at the meeting. "They run into the wall headfirst and run up [it] like nothing happened." He analyzed 79 runs by nine animals, and about 77% of the time, the roaches hit the wall with their head first and pushed themselves up the wall by extending their hind legs. In the other trials, the insects slowed and angled their bodies upward as they approached the wall, taking the brunt of the collision on their front legs. When crashing headfirst, they ran faster—65 to 129 centimeters per second, compared with 54 to 78 centimeters per second when angling up the wall. Those speeds are "the equivalent of a human running 75 to 175 miles [120 to 280 kilometers] per hour into a wall," Jayaram reported.

A cockroach's hard outer skeleton protects its body and brain from injury, enabling it to be imprecise in gauging when to shift from running to climbing. "Small robots [or] animals have key advantages in terms of strength and

collision limits," says Andrew Biewener of Harvard University. There's "no need to be particularly careful about crashing one's head at such a small size."

"What we often perceive as perfection in the movements of animals is not always true when we can make detailed observations," adds Frank Fish of West Chester University in Pennsylvania. "Mistakes are made all the time. ... This study enhances our understanding of reaction time, kinematics, and nervous performance with regard to mistakes involved in collisions."

The results suggest that rather than try to control movements precisely, cockroaches—and possibly other small creatures—depend on the robustness of their bodies or limbs to absorb energy from crashes. "The immediate application of this work is in robotics," says Fish. Robot experts are already taking note, says Full. In the lab of UCB's Ronald Fearing, one 10-centimeter-long robot, called DASH, has an "exoskeleton" that's rigid along some dimensions and flexible along others. It can fall 28 meters and land unscathed. And the École Polytechnique Fédérale du Lausanne has a 37-centimeter-long flying robot called AirBurr that seems a bit clumsy as it maneuvers through an obstacle course toward its target. But because it's covered in a sheath, it quickly recovers from any collisions and continues on its way. The idea is to "evolve a 'smart' body that can manage energy rather than putting all the control in the brain and neural sensors," says Full. As robots get smaller, lighter, and more energy-efficient, "a robust design holds the key." —E.P.

Rattan Stuck in a Growth Mode

Why would a vine grow 100 meters long if it can reach the top of its supporting tree in less than 25 meters? Follow Alice down the rabbit hole to find the answer, says Nick Rowe of the Botany and Computational Plant Architecture Laboratory at the University of Montpellier in France.

Rowe studies how climbing plants attain and maintain their footholds in the forest. In 2003, he and graduate student Sandrine Isnard decided to look into the world's tallest vines, the rattan palms. Rattans dwarf other floral giants: The biggest redwoods top out at 117 meters, but the record rattan palm was measured at 172 meters, says Rowe, with much of the extra stem coiled

on the ground. The stems are widely harvested for cane furniture.

He and Isnard wanted to compare the true rattans, found in Southeast Asia and Africa, with a tall South American vine called *Desmoncus*; the vines look alike and grow similarly although they are not closely related. The researchers also wanted to see how these climbing palms, which belong to a group of plants called monocots and don't lay down woody tissue, grew differently from woody vines, known as lianas.

Climbing plants often undergo a radical change as they grow. When they first sprout, they need to be stiff to grow tall enough to reach the branches or trunk of the tree that will

support them. But once they are entwined among the canopy or lie in coils on the ground, vines need to bend and twist without breaking. Lianas and other woody vines accomplish this transition by changing the type of wood they lay down in their stems. But because monocots don't have woody tissue, they can't adopt that stratagem.

Both rattan palms and *Desmoncus* have modified leaves that bear hooks sometimes arranged around the stem like a grappling hook. Other rattans send out long, unbranched "flagella" with spines that can grab onto nearby vegetation. The stems are surrounded by tubular leaf sheaths from which the hooked leaves and flagella sprout.

Isnard went to China and French Guiana and mechanically tested these climbing



plants to assess the strength of the spines and hooks and the flexibility of stems at different lengths from the ground. She also looked at the cross-sectional geometry of the stems and flagella and compared leaf sheaths across various species.

Tests showed that the leaf sheaths, which can take up 30% to 40% of the stem diameter, are what make the vine stiff, contributing up to 90% of the rigidity.

A climbing palm is stiffest near the top, its growing tip. But 3 to 8 meters below, the leaf sheath degrades and falls off so that the lower stem bends much more easily. In this way, the stem is pliable enough to survive swaying in the attached trees and, lower down, form loops without breaking as it piles up on the ground. "It's a cheap, throwaway method of making a stem flexible," says Rowe. Thus, rattans do what woody plants do—vary the flexibility of their stem along its length—"though employing completely different ways of doing it."

There is a catch, however. As the sheath disintegrates, the hooks and spines are lost, too, so that portion of the stem becomes unhooked. At the meeting, Rowe proposed that this sets up a positive feedback loop, because only by continuing to grow can rattan palms stay attached. In Alice's wonderland, the Red Queen told Alice that she had to keep running just to stay in the same place. The rattans "must continue to grow to stay aloft," says Patrick Martone of the University of British Columbia, Vancouver. "Thus, rattans produce some of the longest stems in the world."

—E.P.

Koalas Calling

It's hard to get a fix on the behavior of animals that sleep most of the time and, when they do wake up, move around primarily in the dead of night. Yet that's what researchers need to do to best protect Australia's cuddly koala, a national icon whose numbers have been dramatically declining in recent years. Fortunately, with a few cell phones and Global Positioning System (GPS) collars, the koala's secretive life is slowly being revealed. During mating season, females make midnight sojourns to visit bellowing males, William Ellis, a terrestrial ecologist at the University of Queensland in Australia, reported at the meeting.

Koalas inhabit woodlands of southern and eastern Australia, often living in urban and agricultural landscapes. They specialize in eating eucalyptus, which tends to be toxic to most animals and barely digestible. Koalas spend about 5 hours a day munching on leaves and most of the rest of the time asleep, digesting their meals.

Males sometimes bellow, emitting a deep groaning noise that lasts about a half a minute, and the conventional wisdom was that their vocalizations are communicating territory information to other males. "But there are no reports that have systematically recorded bellows from within a natural group," says Ellis.

Since 1998, he and his colleagues have been studying koalas at a remote island in the Whitsunday Islands, 1100 kilometers north of Brisbane. In one area, 25 animals wear GPS collars that track their movements, and three cell phones are powered by car batteries attached to solar chargers to listen in on koala conversations. With help from the Queensland University of Technology, the researchers program the phones via text messaging to turn on at specific times to record for certain lengths of time. "The way they have set up the cell phones is very innovative," says Abraham Miller, a behavioral ecologist at the University of Tampa in Florida.

To study bellowing, Ellis's team turned the phones on for 4 minutes every hour and recorded the night sounds. They found that "a lot of the conventional wisdom about koalas didn't stack up," says Ellis.

For one, males were supposed to have larger home ranges than females, but the



Keeping tabs. The collar on this adult koala relays its GPS coordinates to researchers.

GPS tracking revealed that the sexes actually had about the same size territories—important information for conservation planning, Ellis points out. The ranges overlap with a half-dozen other koalas and tend to shift in location over time; but no two koalas wind up in the same tree at the same time, Ellis reported. "This suggests koala behavior is much more complex than previously thought," says Miller.

Male and female activity was similar, except that sometimes during the mating season, a female would abruptly leave her tree, head off, and then usually return several hours later.

The cell phones indicated that bellowing takes off right before the breeding season, October through February, and most often occurs at about midnight. When the researchers matched the GPS and sound data, they realized that males don't move in response to bellows. However, "unusually large movements from females occur when we also hear most bellows," says Ellis, "so we think that the females are responding." He is now comparing females' excursions with the timing of conception to see if indeed the bellow is a mating call. Miller would like to see more koalas included in the study. But he says Ellis is on the right track: "It is necessary to have an understanding of [koala] life history as well as behavior to effectively manage and protect the species."

—ELIZABETH PENNISI



Slippage. As rattans shed leaves and hooks, flexible lower stems slip away from the tree.

CREDITS (TOP TO BOTTOM): PHOTO BY FRED BERCOVITCH; NICK ROWE

SCIENCE AND SOCIETY

Lights! Camera! Science?

Science film festivals are popping up around the world. But does *Avatar* belong on the same screen as a documentary about stem cells?



BORDEAUX, FRANCE—A slimy green ectoplasm gobbles up all the food and drink at a swanky hotel. A giant marshmallow dressed as a sailor lumbers through the streets of New York City. *There's something weird, and it don't look good. Who ya gonna call?*

Ghostbusters! of course.

Even a quarter-century after it filled cinemas, and its irresistible theme song hit number one, the zany film about three failed parapsychology students and their ghost extermination service is still fun. But the movie's 4 a.m. screening at a multiplex here in December did raise a question: Why was it part of a festival for *science* films? The organizers of Cinémascience, a festival now in its second year, admit they just didn't put the bar for scientific content all that high. *Ghostbusters* slipped into the program as part of an all-nighter of sci-fi classics.

Cinémascience is part of a new wave of film festivals around the world that show

nothing but science-related films and claim growing audiences every year. Bangkok, Athens, Paris, and New York City have all seen the birth of such festivals in the past 5 years. A few others—such as the Milan science film festival and Australia's Scinema, which runs on 200 screens across the country—have been around for a decade.

But check out the programs of each, and you discover that they have radically different ideas about what constitutes a science film. "I've been asked that question over and over, and I still don't have a good answer," says cell biologist-turned-filmmaker Alexis Gambis, director of the Imagine Science Film Festival in New York City, launched in 2008. Consequently, you can go to three different festivals and have three very different experiences.

The organizers say the festivals exist in part because there's so little science on the big screen. Sure, a good portion of Hollywood's

biggest moneymakers are science-fiction films, and there's no reason why record-smashing *Avatar*—whose alien world was shaped with advice from plant biologists and linguists—can't be called a science film, says Emory University physicist Sidney Perkowitz, author of *Hollywood Science: Movies, Science, and the End of*

the World. But the movie industry is far less interested in films about the lives and work of scientists and in scientific documentaries, rare blockbusters such as *A Beautiful Mind* and *March of the Penguins* notwithstanding.

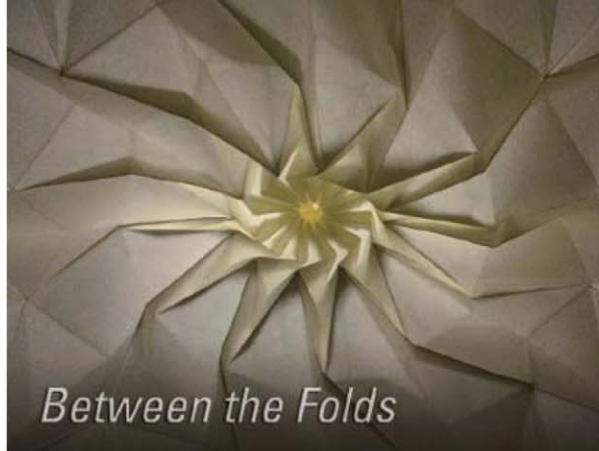
And yet, with the advent of the digital camera and video-editing software, almost anyone can make a film, and many scientists, former scientists, and students are becoming part-time directors. Pariscience, an annual film festival in the French capital, now receives some 300 submissions, says director Jean-Pierre Gibrat. Festivals' main headache today is not to find films but to break even, especially because some don't charge admission. Most rely on sponsors; Scinema is underwritten and organized by CSIRO, Australia's national science agency, for instance, and Imagine has many sponsors, including *Science*.

Some festivals clearly focus on education. The festival in Bangkok tries to spark Thai children's fascination for science by showing "family edutainment"—that is, short documentaries on science and nature made for youngsters. Most of the 45 films shown at the 2009 version were originally TV programs made elsewhere in the world and then dubbed in Thai. More than half were German and French; not a coincidence, because the festival was organized by the Goethe Institute—which tries to spread German culture—and the French Embassy in Bangkok.

Other festivals cater to children as well, but not exclusively. The one in Milan, organized by the University of Milan's physics department, shows films for 11- to 18-year-olds in the mornings; afternoons are for documentaries for adults and the official competition; and evenings are given over mostly to historical films, with the occasional drama or science-fiction film thrown in. The 2009 edition featured *Inherit the Wind*, a 1960 courtroom drama about the Scopes Monkey Trial, as well as *2001: A Space Odyssey*.

The festival in Bordeaux, organized by France's National Centre for Scientific Research (CNRS), is unique because it shows only feature-length fiction films. That's in part to avoid competing with Pariscience, which has only documentaries. "We don't want to step on anybody's toes," says Cinémascience programmer Denise Anderson. But because science-based fiction films are quite rare, Cinémascience's program is thinner on actual science than most others. One of the two winners of a Jury Award was *Skin*, a gripping drama based on the life of Sandra Laing, a black woman born to white parents in South Africa in the 1950s. Except for the brief appearance of a geneticist who explained in a courtroom that "black" genes can lurk in





Leonardo da Vinci's dream of flying, a rap video about the polymerase chain reaction, and *Skylight*, a "mock animated documentary about the ecological plight of penguins in the Antarctic." (For a review of the festival, see *Science*, 4 December 2009, p. 1348.) Gambis has plans for satellite events in London, Paris, and South America.

white families, science played almost no role in the film.

Cinémascience's philosophy is that each film serves as a springboard for a debate between filmmakers, the audience, and CNRS researchers, says Anderson. After *Skin*, anthropologist Gilles Boëtsch and director Anthony Fabian discussed racism and the lack of science behind the concept of race. With that criterion, however, it appeared that almost any film could be called a science film. The screening of *Admiral*, a lavishly produced \$20 million Russian drama about the 1917 revolution, was followed by a debate with two CNRS experts on Russian history—but even director Andrei Kravchuk was surprised that his movie had been selected for a science film festival. The formula seems to work, however. Attendance rose from about 6000 last year to 8000 in 2009.

New York City's Imagine festival is similar to Cinémascience, says Gambis, in that it "tends to encourage a story, a narrative," while shunning straight-up documentaries. "The goal is not to teach or lecture people," says Gambis. "We wouldn't show 2 hours about antibiotics or a film about how stem cells work." Instead, Imagine in 2009 featured an animation about

Stressing narrative and showing fiction does raise a perennial question among science film buffs, however: Should the science be accurate? Yes, says Gambis—his festival even has a special award for scientific accuracy, sponsored by *Nature*. And scientists tend to agree. At Cinémascience, CNRS robotics researcher Agnès Guillot was taken aback by *Surrogates*, a sci-fi thriller in which people stay at home while robots resembling them and controlled by their minds go out in the real world.

Perkowitz argues that accurate science often gets in the way of a good story. He says directors should be allowed "one major suspension of disbelief per film," such as travel faster than the speed of light. At festivals especially, even imperfect science can make for great debates, Perkowitz says. Indeed, his ideal festival would consist of a couple of stunning documentaries, debates with scientists—and a good dose of razzle-dazzle sci-fi films, complete with laser guns and planets being blown up. "I have no doubt that some 12- or 14-year-old kids will become scientists because something in those films triggers their imagination," he says. "It's what happened to me."

—MARTIN ENSERINK



A sampling of the films and directors that took the honors at science film festivals around the world in 2009.

Scinema, Australia

Best Director

400 Years of the Telescope

By Kris Koenig (U.S., 60 min.)

Documentary about the history of the telescope since Galileo.

Best Film

Between the Folds

By Vanessa Gould (U.S., 56 min.)

Documentary exploring the art and science of origami.

Science Film Festival, Thailand

Jury Award

There's Something About Species

By Denis van Waerebeke (France, 82 min.)

Documentary exploring the tree of life and the history of evolution.

Imagine Science Film Festival, New York City

Audience Award

Leonardo

By Jim Capobianco (U.S., 9 min.)

Animation in which Leonardo da Vinci tries to realize his dream of flying.

Scientific Merit Award

Magnetic Movie

By Ruth Jarman and Joe Gerhardt (U.K., 5 min.)

Documentary that visualizes magnetic fields in bright colors.

Cinémascience, Bordeaux

Jury Awards

Skin

By Anthony Fabian (U.K./South Africa, 107 min.)

Biographical drama about a black girl born to white South Africans in the 1950s.

Dirty Mind

By Pieter Van Hees (Belgium, 102 min.)

Comedy about a stuntman who undergoes a radical personality change after suffering brain damage.

Audience Award

The Stranger in Me

By Emily Atef (Germany, 99 min.)

Drama about a woman who suffers from severe postpartum depression.

LETTERS

edited by Jennifer Sills

Stop Listening to Scientists?

AS A CLIMATE SCIENTIST AND A CONTRIBUTING AUTHOR TO THE INTERGOVERNMENTAL PANEL on Climate Change, my heart always warms when I hear policy-makers refer to doing what “the science dictates,” as President Obama did in his remarks toward the end of the U.N. Climate Change Treaty negotiations in Copenhagen, Denmark. However, after the first-hand experience of the rapid crash of the Copenhagen meeting, I have changed my thinking: World leaders, please stop listening to us! I don’t say this because I have lost faith in the verity of scientific results or the projected warming and subsequent global damages. I say this because international policy-makers are adhering too rigidly and too literally to recommended concentration thresholds and emissions targets, and it is crippling the international policy process.

By demanding nothing less than rigid recipes, we have lost valuable momentum. To combat this trend, I offer the following recommendations.

Leave aside the near-obsessive need to benchmark everything against the 2°C target. Science has done a commendable job outlining the boundaries of the climate change problem, and those boundaries are well-considered, rigorous guideposts, but don’t use science recommendations as a litmus test for policy success or failure.

Don’t let the perfect be the enemy of the good. Accept any binding commitment as long as it demonstrates effort beyond Kyoto or “business as usual” (whichever requires the greater effort). This can be tightened in the future—you can’t amend something you don’t have.

Lower the rhetoric. Climate politics has evolved to a point where if one side thinks the other side isn’t listening, they shout louder and invoke phrases like “genocide” and “murder.” Overblown rhetoric inevitably leads to the well-known “donor fatigue.”

Other than commitments to slow deforestation and forest degradation, leave forestry complications out of a current agreement. It has generated confusion, raced ahead of science, opened mitigation loopholes, and consumed far too much negotiating oxygen.

To the developing world: Approach funding offers as a starting point to get a funding system flowing. You can’t attract new revenue, or extend or add funds, to financing that doesn’t exist.

Agree to even loose commitments on monitoring, reporting, and verification (MRV), a key sticking point in the Copenhagen talks. Science can solve this problem, but can’t get started without a clear signal and research commitment from all large emitting countries.

Prioritize country commitments to mobilize domestic and international energy research support. In addition to technology transfer opportunities, effort can be directed toward MRV.

In short, we need agreement, even an imperfect agreement, to show a consistent and committed forward momentum. What were general scientific guideposts have become ossified deal-breakers. Instead, we need a sufficient signal to unleash the private and public resources to begin decarbonization. With that, we will start walking in the direction of our goal but leave ourselves open to shortcuts we can’t see at the outset.

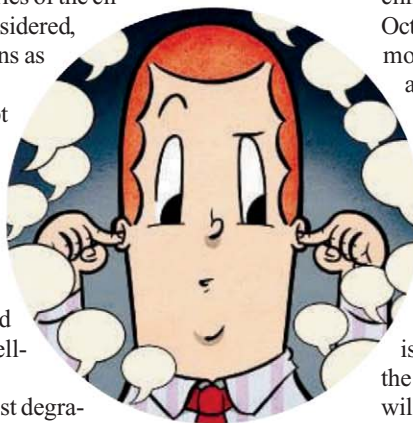
KEVIN ROBERT GURNEY

Department of Earth and Atmospheric Science, Purdue University, West Lafayette, IN 47907, USA. E-mail: kgurney@purdue.edu

Carbon Calculations
to Consider

T. SEARCHINGER *ET AL.* (“FIXING A CRITICAL climate accounting error,” Policy Forum, 23 October 2009, p. 527) suggest that it would be more scholarly to account for all carbon assimilation and release as function of time rather than just consider biomass as carbon neutral. However, they do not distinguish between felling mature forest to get wood for burning and doing so to grow energy crops. What matters is what is done with the wood from clearing. If combusted, this is a one-time, negative impact on the carbon balance. If the wood is used for house construction or furniture, the immediate climate impact is zero. There will be a negative impact only if these items are later burned.

Some of the same authors recently attacked “second-generation” biofuels (1), making the prediction that biofuels will soon be derived entirely from cellulosic material grown on marginal land. This is at variance with another definition of second-generation biofuels: vehicle fuels derived entirely from residues from already-existing biomass cultivation. These are evidently carbon neutral, and no energy inputs are changed. The soil-nutrient balance can be improved because residues from fuel production can be returned to the fields, where they are less likely to foul waterways than are chemical fertilizers. One should remember that most agri- and silvicultural residues are



Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 3 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

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Explaining
ice age cycles

790



Where chromosomes
cross over

791

already used today without recycling nutrients, primarily for power and heat plant combustion. The most sustainable option available to us is to use second-generation biofuels along with recycling.

BENT SØRENSEN

Department of Environmental, Social, and Spatial Change, Roskilde University, DK-4000, Denmark. E-mail: boson@ruc.dk

Reference

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Response

OUR POLICY FORUM POINTED OUT A MAJOR flaw when treaties and laws treat all bioenergy as carbon neutral regardless of the source of the biomass. Sørensen's main point appears to be that when mature forests are cleared to plant bioenergy crops, any use of harvested wood to add to the stocks of long-lived wood products preserves some of the carbon and therefore reduces the scope of emissions. We agree. This statement supports our central point that proper accounting of biomass must reflect its effects on carbon stocks and flows.

Unfortunately, using harvested wood for long-lived furniture or housing does not by

itself make biomass carbon neutral. When forests are cleared and planted with energy crops or plantation forests, much carbon is lost to the atmosphere from unharvested branches, roots and soils. In addition, many mature forests continue to sequester carbon if left standing (1), and the loss of this ongoing carbon sequestration counts as another carbon cost of clearing the forest. Forest regrowth or the displacement of fossil fuels through energy crops provides carbon savings, but analyses that count the amount of harvested wood incorporated into furniture and houses still find that energy crops can take more than 100 years to repay the carbon debt from clearing forests (2). Given the urgency of reducing greenhouse gas emissions (3), policy should focus on the net effect on emissions over a few decades at most. Over these periods, forest clearing for energy will rarely, if ever, approach carbon neutral outcomes—i.e., cause a 100% reduction in carbon emissions compared to use of fossil fuels. In practice, carbon emissions will often increase (4).

Rather than attacking second-generation biofuels, we have distinguished biofuels in both our recent and previous papers based

on the feedstock. The key distinction is whether biofuels merely shift carbon from one use to another, which does not reduce greenhouse gases, or instead result in “additional” carbon uptake in plants or reduced biomass decomposition, both of which can reduce greenhouse gases. Whether crop and timber residues are better used for liquid biofuels or electricity and power is a separate question. Many studies have found that using biomass to replace fossil fuels for electricity or power saves more carbon than using biomass for liquid fuels (5, 6). One reason is that much of the energy in biomass is lost in the process of transforming it into a liquid fuel (7). Sørensen is correct that such comparisons need to consider the potential benefits of by-products that can displace synthetic fertilizers. Most biofuels do not, and probably will not, produce such by-products. The potentially expanded use of biomass for a wide range of industrial applications highlights the importance of correct accounting.

TIMOTHY D. SEARCHINGER,^{1*}

STEVEN P. HAMBURG,² JERRY MELILLO,³

WILLIAM CHAMEIDES,⁴ PETR HAVLIK,⁵

DANIEL M. KAMMEN,⁶ GENE E. LIKENS,⁷

MICHAEL OBERSTEINER,⁵

MICHAEL OPPENHEIMER,¹ G. PHILIP ROBERTSON,⁸

WILLIAM H. SCHLESINGER,⁷ G. DAVID TILMAN,⁹

RUBEN LUBOWSKI¹⁰

¹Woodrow Wilson School, Princeton University, Princeton, NJ 08544, USA. ²Environmental Defense Fund, Boston, MA 02108, USA. ³The Ecosystems Center, Marine Biological Laboratory, Woods Hole, MA 02543, USA. ⁴Nicholas School of Environment, Duke University, Durham, NC 27708, USA. ⁵International Institute for Applied Systems Analysis, Laxenburg 2361, Austria. ⁶Energy and Resources Group, University of California at Berkeley, Berkeley, CA 94720, USA. ⁷Cary Institute of Ecosystem Studies, Millbrook, NY 12545, USA. ⁸WK Kellogg Biological Station, Michigan State University, Hickory Corners, MI 49060, USA. ⁹Department of Ecology, Evolution and Behavior, University of Minnesota, St. Paul, MN 55108, USA. ¹⁰Environmental Defense Fund, Washington, DC 20009, USA.

*To whom correspondence should be addressed. E-mail: tsearchi@princeton.edu

CORRECTIONS AND CLARIFICATIONS

Reports: “Plumage color patterns of an extinct dinosaur” by Q. Li *et al.* (published online 4 February in *Science Express*; 10.1126/science.1186290). In the abstract view, Long Miao was mistakenly included as an author. The author list on the PDF file is correct.

Reports: “Elevated CO₂ reduces losses of plant diversity caused by nitrogen deposition” by P. B. Reich (4 December 2009, p. 1399). The 0.70 and 0.65 values and corresponding <0.0001 values in the first row of Tables 1 and 2, respectively, are the R² values and significance values for the whole models (and should not have been placed in the columns labeled “F value” and “P > F,” respectively).

Research Articles: “The pelvis and femur of *Ardipithecus ramidus*: The emergence of upright walking” by C. O. Lovejoy *et al.* (2 October 2009, p. 71; full text online only at <http://dx.doi.org/10.1126/science.1175831>). The first sentence of the Fig. 4 caption should read, “Fig. 4. Lateral (A) and posterior (B) CT scan surface renders of (Left) MAK-VP-1/1 (*Au. afarensis*, cast) and (Right) ARA-VP-1/701 (*Ar. ramidus*, original).”

Research Articles: “Reexamining human origins in light of *Ardipithecus ramidus*” by C. O. Lovejoy *et al.* (Special Section on *Ardipithecus ramidus*, 2 October 2009, p. 74; full text online only at <http://dx.doi.org/10.1126/science.1175834>). The article notes that daily sperm production per gram of testes in humans is <0.06 × 10⁶. The correct figure is <6 × 10⁶.

Reports: “Reversible reactions of ethylene with distannynes under ambient conditions” by Y. Peng *et al.* (25 September 2009, p. 1668). The Figure 2 legend incorrectly stated that average bond angles were measured in degrees Celsius. The bond angles were actually measured in degrees.

Reports: “Allelopathy and exotic plant invasion: From molecules and genes to species interactions” by H. P. Bais *et al.* (5 September 2003, p. 1377). The authors have had difficulty replicating the high and consistent levels of catechin found in soils surrounding *Centaurea maculosa* plants as originally reported (see Fig. 1A). For the most part, catechin seems to be present in low concentrations or not detectable in soils surrounding *C. maculosa* in the field, with the exception of one sampling period during which it was found at concentrations similar to those in the Report [see L. G. Perry, *J. Chem. Ecol.* **33**, 2337 (2007)]. Furthermore, in vitro exudation of catechin by *C. maculosa* roots has not been reproducible by the Vivanco laboratory, and therefore the origin of catechin in the field is unclear. However, the authors have reconfirmed that catechin has the signaling and phytotoxic activities indicated in the Report.

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MEDICINE

Reading Your Genes

Tim Harris

Everyone is interested in heredity, whether they know what it means or not. As Sydney Brenner likes to say, everyone has an Uncle Fred who smokes like a chimney, drinks like a fish, never exercises, and lives to be 95. Unfortunately everyone also knows someone who died much too young from cancer or heart disease. People are generally curious to know why that is, and they are aware that it has something to do with their genes.

In *The Language of Life*, Francis Collins considers that something, discussing how heredity works and how our genes, in complex association with the environment, influence our health. Collins, the director of the U.S. National Institutes of Health, offers clear explanations of nearly all the technologies that are driving the personal medicine revolution, from traditional linkage genetics to second-generation DNA sequencing and genome-wide association studies. He draws on considerable personal experience, gained in his own laboratory, which was involved in the discovery of genetic defects causing cystic fibrosis, neurofibromatosis, and other disorders. His account is also influenced by his experiences leading the Human Genome Project (recounted in a short appendix) and all the attendant publicity.

Collins clearly explains the science of personal genetics through examples that are easy to relate to and to understand, such as the single-gene disorders phenylketonuria, sickle cell disease, long QT syndrome, and Tay-Sachs disease. (Appendices provide a glossary of genetic terms and a short sketch of how genetic inheritance works that are also helpful.) Early in the book, he includes a valuable explanation of the meaning of relative risk that helps the reader to understand the importance of many of the subsequent examples in which multiple genes determine susceptibility. He also, appropriately, mentions the “dark matter of the genome,” an understanding of which may help answer

the question of the missing heritability of diseases in the results of genome-wide association studies.

As the subtitle suggests, Collins pays particular attention to the potential practice of personalized medicine and covers all the relevant ground. He elegantly discusses somatic (e.g., *KRAS*) and germline (e.g., *BRCA1*) oncogene mutations, which predispose one to cancer, and the differences and similarities between them. Using the discovery of Gleevec (for the treatment of chronic myelogenous leukemia) as the paradigm, Collins explains how such knowledge is changing therapy. He also describes the importance of changes in drug-metabolizing enzymes that affect the efficacy or side-effects profile of many drugs, including those involved in the metabolism of warfarin and tamoxifen.

With the author's academic pedigree and prowess, it is hardly a surprise that he gives relatively short shrift to the importance of the commercial sector in translating these DNA-

driven discoveries into diagnostic or therapeutic practices that benefit patients. The use of antiretrovirals for HIV and monoclonal antibodies and tyrosine kinase inhibitors for cancer has fundamentally changed treatments, and without the commercial sector many of the discoveries so elegantly described by Collins just would not be available in a timely fashion to the patients who need them. Such translation to the clinic cannot be done properly without an effective commercial

interface, and it is a pity that Collins did not take the opportunity to make this point. Drug discovery, although seemingly a straightforward process (one outlined in another appendix), is very hard; the impression that it can be carried out just as well by the academic community is misleading. It is the symbiosis of industry and the academic world that matters. Nor will gene therapy provide an alternative solution either any time soon—at least not for most common diseases.

Nevertheless, the book gives direct-to-consumer genetics companies (also known as personal medicine companies) such as 23andMe, deCODEme, and Navigenics frontline billing—rather more than is appropriate from the accuracy and utility of the data that can currently be derived from personal DNA sequence information. In this context, Collins's discussion of ApoE alleles and the risk of developing Alzheimer's disease is useful: even if one has the “wrong” alleles (here, two copies of ApoE $\epsilon 4$), there is not much one can do about the disease's onset beyond perhaps a few mind exercises.

But it is justified to talk about genetics companies, given that within the next five years genome sequence information will be readily available for many people for under \$1000. Besides, doing so makes for interesting and entertaining reading. The hope and expectation is that our biological comprehension of sequence information will keep up with our increasing ability to generate genome- or exome-wide DNA sequences.

The Language of Life provides the interested reader (professional or lay) an accurate snapshot of our current understanding of human genetics as it relates to personal health and well-being. As Collins himself says, by the time you have read the book you

The Language of Life
DNA and the Revolution
in Personalized Medicine

by Francis S. Collins

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The reviewer is at SAIC Frederick, Incorporated, Post Office Box B, Frederick, MD 21702, USA. E-mail: harristjr@mail.nih.gov

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will probably know more than your physician about genetics and health. So to avoid such an indictment, physicians had better read the book as well. Each chapter ends with a checklist of things, from examples just discussed in it, that one can do to live a healthier life through better understanding of one's own genetics. How much of it all will be used by Uncle Fred and his offspring is a little more difficult to determine.

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HISTORICAL FICTION

Neptune's Daughter

Richard Milner

In *The Coral Thief*, Cambridge-based author Rebecca Stott has created an intricate, suspenseful burglary caper that combines mystery, romance, and cloak-and-dagger intrigue—all set against a background of the developing life sciences in post-Napoleonic Paris. An inventive and versatile writer, Stott is perhaps best known for her admirable biography, *Darwin and the Barnacle (1)*, in which she charmed readers as both an accomplished storyteller and a rigorous historian of science.

Stott's narrator and protagonist, Daniel Connor, an ambitious British anatomist, is drawn into the web of the beautiful and brilliant Lucienne Bernard. An aristocrat who narrowly escaped the guillotine during the Revolution, she now survives by cunning in a shadowy, marginalized Parisian underground of rebellious intellectuals and wanted criminals. Connor first encounters Lucienne during a coach ride. When he falls asleep, she steals his letter of introduction to Professor Georges Cuvier, along with some rare corals and fossils intended for the great man. After several attempts to retrieve his property, Connor is seduced by this mysterious miscreant—not only by her beauty and wit but also by her passion for the new insights that are brewing within the French scientific establishment.

Stott provides a vivid depiction of how this ongoing revolution in thinking about Earth's age and the nature of species became entwined with the politics of the day. Lucienne knows students of Lamarck, "the trans-

formist professor at the Jardin des Plantes," whose ideas leapt beyond science to shape a new generation's world view. Everything was moving forward (*la marche*), leaving the past behind. Stott intersperses her story with wonderfully evocative vignettes of Napoleon's final voyage to his exile at St. Helena, where he must leave his own glorious past behind.

In Lamarck's theory of "transformation," some students saw "a bloody brilliant dethronement of man.... [W]e're just one more organism among all those others.... The *people* will make the future, not the kings anymore." The zoologist Daubenton, according to Stott, remarked in a lecture that "the lion could no longer be called the king of beasts because there were no kings in nature," and his audience cheered.

Cuvier, who holds the key to Connor's career, was the king of French natural history. Nothing could dislodge him from his perch at the top of the French scientific establishment—not rivals, revolutions, emperors, nor the restored Bourbon king. With the help of scores of students and assistants, he produced his multi-volume, illustrated compendium of every known animal. He taught that species were immutable and that Earth was 6000 years old, shaped by a series of "catastrophes" every few thousand years.

Lucienne tells Connor that Cuvier is wrong about species being fixed and about Earth's age. She notes that "some [coral] reefs are a thousand feet thick," which at a growth rate of even an inch per year implies an age of about 12,000 years. (Modern estimates for many reefs are closer to 0.125 inch per year.)

Stott's picture of the development of natural science as it was intertwined with social and political movements is entertaining and enlightening. However, various inaccuracies of period and possibility, small and large, creep in to disrupt credibility. For instance, she has Cuvier deliver an impromptu lecture during which he is handed a pair of elephant skulls and effortlessly lifts one high into the air. (The skull would have weighed more than a hundred pounds, even without tusks.) But that's only a minor glitch. Unfortunately, the novel's central conceit disrupts the integrity of her chosen historical time frame. It is limned in the beautifully written final paragraph:

[Lucienne] had seen Red Sea coral spawn, she said. When the sea reaches the right temperature, when they are ripe, when the moon reaches a certain point, just once

a year, down there on the coral reefs, the dark waters explode into white smoke clouds.... The fishermen say it's the moon that makes them spawn, she had said, and I said: How can they see the moon? They have no eyes. Perhaps they have other ways of seeing and knowing, she had said. Perhaps we all do. There's a grandeur in that.

This romantic coda, evoking both the mysteries of marine fecundity and "grandeur" of nonscientific ways of knowing (using one of Darwin's favorite words to suggest the opposite of his meaning), calls up a triumphant symphony of nature, forever eluding explanation by the human intellect—at least from an 1815 perspective.

It might seem reasonable that these periodic blizzards of sperm and eggs would have posed an awe-inspiring mystery down through the centuries, but mass spawning of corals was unknown in Cuvier's day. In fact, the corals' late-night reproductive extravaganzas were first documented in 1981 (2). More recently, researchers have shown that the polyps' cryptochromes (light-sensitive cells that perceive blue light) tell them when to release their trillions of gametes: they do "see" the Moon after all (3).

Young Darwin, whom Stott mentions on her last page as the harbinger of things to come in science, became fascinated with corals during the *Beagle's* stop in the Cocos Islands. He noted in his journal that although the monumental undersea "mountains of stone accumulated by the agency of various minute and tender animals" are an impressive sight, they are still more wonderful, on later reflection, to "the eye of reason" (4). In Darwin's view, that's where the grandeur comes in.

As she has shown in her well-received previous novel *Ghostwalk* (5) and expressed in interviews, the author really wants to have it both ways. Her own upbringing (like Lucienne's) veered from an extreme anti-science religiosity to a burgeoning Enlightenment rationality. Like her characters, Stott vacillates between being a mystic and being a scientific thinker, as do we all. Despite the anachronisms in her history of reef biology, her poetic and entertaining tale illuminates the delicate boundary that separates both ways of knowing nature.

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The reviewer is the author of *Darwin's Universe: Evolution from A to Z*. Web site: <http://darwinlive.com/>. E-mail: rmilner@nyc.rr.com

Sustainability and Global Seafood

Martin D. Smith,^{1,2*} Cathy A. Roheim,³ Larry B. Crowder,⁴ Benjamin S. Halpern,⁵ Mary Turnipseed,¹ James L. Anderson,³ Frank Asche,⁶ Luis Bourillón,⁷ Atle G. Guttormsen,⁸ Ahmed Khan,⁹ Lisa A. Liguori,¹⁰ Aaron McNevin,¹¹ Mary I. O'Connor,⁵ Dale Squires,¹² Peter Tyedmers,¹³ Carrie Brownstein,¹⁴ Kristin Carden,¹⁵ Dane H. Klinger,¹⁶ Raphael Sagarin,¹⁷ Kimberly A. Selkoe^{5,18}

Although seafood is the most highly traded food internationally, it is an often overlooked component of global food security. It provides essential local food, livelihoods, and export earnings. Although global capture fisheries production is unlikely to increase, aquaculture is growing considerably. Sustaining seafood's contributions to food security hinges on the ability of institutions, particularly in developing countries, to protect and improve ecosystem health in the face of increasing pressures from international trade.

Seafood (fish and shellfish harvested from capture fisheries and aquaculture production in marine and freshwater environments) contributes at least 15% of average animal protein consumption to 2.9 billion people and as much as 50% for some small island and West African states (1). Seafood is the main source of omega-3 fatty acids that are essential for brain development (2) and provides important micronutrients for the poor (3). As a source of livelihood, capture fisheries and aquaculture employed 43.5 million people in 2006, and 520 million people relied on income from seafood production (1). Seafood is also the most highly traded food commodity internationally (1). Fish and shellfish exports from developing countries exceed the value of coffee, rubber, cocoa, tea, tobacco, meat, and rice combined (1). Developing countries benefit from this trade by exporting high-valued seafood to developed countries, importing low-valued seafood, and using the surplus value to purchase other goods and services (fig. S1). However, they often lack the institutions necessary to prevent deleterious ecosystem

impacts of seafood production and to sustain trade benefits. Developed countries have a history of these problems, as well, but with less-obvious consequences.

Although terrestrial food systems provide protein, support livelihoods, and generate export earnings, two characteristics of fisheries and aquaculture production uniquely threaten food security: tight coupling to ecosystems and dependence on common-pool resources. Fisheries and aquaculture are vulnerable to exogenous shocks to ecosystems such as climate change, but endogenous changes are particularly important. Common-pool fish stocks are often open-access, and fishing effort can push stock levels beyond maximum sustainable yield. In those cases, price increases lead to reduced seafood production (4, 5). This scenario does not generally occur in terrestrial food production.

Fishing not only reduces target species populations but also can alter marine food webs (6) and has cumulative impacts on marine ecosystems (7), undermining the productive capacity of fisheries. Ultimately, the total productivity of a capture fishery is limited by the target species' ability to reproduce, and poor governance often leads to fish populations being pushed beyond this limit.

Aquaculture attempts to decouple fish production from environmental fluctuations by controlling growing conditions, feed input, and disease (8, 9). However, poor management can lead to reduced production even when prices rise, partly due to poorly defined property rights in locations where aquaculture is conducted. In estuarine and marine environments, nutrient pollution, farmed fish escapes, disease spread, and the use of cap-

Tight coupling to ecosystems and dependence on common-pool resources threaten fisheries and aquaculture.

ture fish in feed also threaten aquaculture's sustainability (10).

Consumption is not shared equally among countries (see the figure on page 785). Levels are high in developed and island countries but low in some developing countries (China and Southeast Asia are notable exceptions). Overlaying net exports, governance, and undernourishment suggests that seafood's contribution as a source of protein and livelihood is precarious. To compare institutional effectiveness across countries, we used an average of four governance indicators developed for the World Bank (11) as a proxy. Countries with undernourishment and weak governance often serve as net exporters of seafood to well-nourished countries with strong governance (see the table on page 786). However, the largest seafood net exporters (China, Norway, and Chile) have neither the weakest governance nor the greatest undernourishment, suggesting that they have some institutional capacity to promote sustainability (see the figure).

At the global scale (see the table), regions with low undernourishment are net importers of seafood from regions with high undernourishment. In principle, developing countries could consume more seafood simply by exporting less of it. But prevailing conditions in the global seafood market make it advantageous for many countries to be seafood exporters and generate surplus value (fig. S1). A population-weighted average governance score follows the same trend as per capita seafood consumption; regions with more undernourishment tend to have weaker governance (see the figure and the table). Poor governance ultimately squanders seafood availability, for example, by failing to control overfishing and bycatch, as well as failing to regulate the environmental impacts of aquaculture. Corruption (included in governance) can also prevent export earnings from benefiting the poor.

On each continent, the governance index is lower in less-nourished regions. Per capita seafood consumption follows the same pattern, except in Oceania, which has a preponderance of small island nations with abundant seafood sources (see the table).

Asia generates most of the world's net seafood exports from countries with moderate to

¹Nicholas School of the Environment, Duke University, Durham, NC 27708, USA. ²Department of Economics, Duke University, Durham, NC 27708, USA. ³Department of Environmental and Natural Resource Economics, University of Rhode Island, Kingston, RI 02881, USA. ⁴Center for Marine Conservation, Nicholas School of the Environment, Duke University, Beaufort, NC 28516, USA. ⁵National Center for Ecological Analysis and Synthesis, University of California, Santa Barbara, Santa Barbara, CA 93101, USA. ⁶Department of Industrial Economics, University of Stavanger, Stavanger, 4036, Norway. ⁷Comunidad y Biodiversidad, A.C. (COBI), Boulevard Agua Marina 297, Colonia Delicias, Guaymas, Sonora, 85420, México. ⁸Department of Economics and Resource Management, Norwegian University of Life Sciences, 1432, Aas, Norway. ⁹International Coastal Network, Department of Geography, Memorial University, St. John's, Newfoundland A1B 3X9, Canada. ¹⁰Marine Extension Service, University of Georgia, Brunswick, GA 31520, USA. ¹¹World Wildlife Fund, Washington, DC 20037, USA. ¹²Southwest Fisheries Science Center, La Jolla, CA 92037, USA. ¹³School for Resource and Environmental Studies, Dalhousie University, Halifax, Nova Scotia, B3H 3J5 Canada. ¹⁴Whole Foods Market, Austin, TX 78703, USA. ¹⁵Department of Ecology, Evolution, and Marine Biology, University of California, Santa Barbara, Santa Barbara, CA 93106, USA. ¹⁶Emmett Interdisciplinary Program in Environment and Resources, Stanford University, Stanford, CA 94305, USA. ¹⁷Institute of the Environment, University of Arizona, Tucson, AZ 85719, USA. ¹⁸Hawaii Institute of Marine Biology, Kaneohe, HI 96744, USA.

*Author for correspondence: E-mail: marsmith@duke.edu

severe undernourishment. China, Indonesia, Vietnam, Thailand, Taiwan, India, and Myanmar are large net exporters (>300,000 metric tons) and, with the exception of Taiwan (for which data are unavailable), have moderate to high undernourishment. China illustrates the potential for aquaculture to contribute to food security by expanding export-oriented and domestically consumed aquaculture. This growth contributed to China's recent substantial reduction in undernourishment (12). Ninety-two percent of global animal aquaculture production occurs in developing countries, of which 31% is carp that is mostly grown in small Chinese facilities for domestic consumption (13). In contrast, Japan is the world's largest net importer (3.82 million

metric tons) and has low undernourishment.

In Africa, severely undernourished regions, e.g., Namibia and Senegal, are net exporters, but moderately undernourished regions are net importers, e.g., Nigeria (see the table). Small amounts of exports from Africa also reflect access agreements between countries in West Africa and other regions (mostly Europe and Asia) to exploit their offshore fish stocks. These landings are counted neither as African production nor as African exports, although they come from African waters.

The United States and European Union countries are well nourished and among the largest net importers. In contrast, large-scale aquaculture production creates opportunities for countries with all levels of nourishment

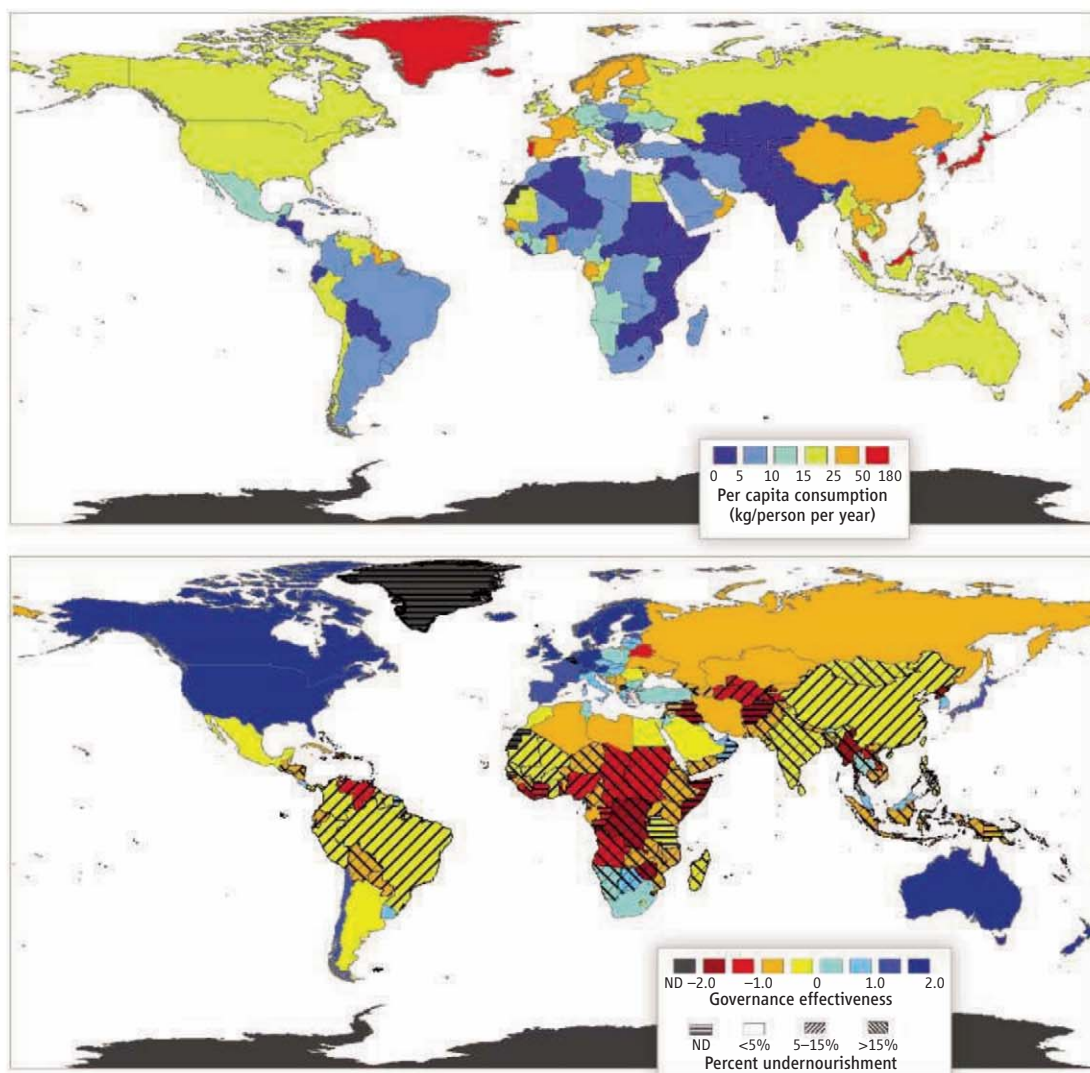
(low, moderate, and high) to be net exporters, e.g., Vietnam, Chile, and Norway.

These data highlight the benefits of the seafood trade but also seafood's precarious role in contributing to food security. Weak governance threatens countries' abilities to consume seafood domestically or export it and use the trade system to purchase other foods. Because much of the world's seafood production comes from regions with weak governance, improved governance is essential to sustain or increase seafood's contribution to food security.

Two very different histories of fish production in Chile and Mexico illustrate the importance of effective governance. Chile's rugged coastline is well suited to salmon farming.

Salmon production has been primarily an indirect source of food through earnings and employment. Global trade and lax environmental regulations in Chile facilitated rapid expansion of salmon farming, but currently the industry is experiencing its worst disease crisis ever, an outbreak of infectious salmon anemia. Although 670,000 metric tons were produced in 2008, the prediction is that Chile will produce less than 100,000 metric tons in 2010. The outbreak has been attributed to institutional failure to react to known risks from lake-based smolt production and unvaccinated fish (14). Chile's crisis tells a cautionary tale about expanding aquaculture production without effective institutions to protect the environment.

The spiny lobster fishery (*Panulirus interruptus*) along the central west coast of the Baja California peninsula is the largest lobster fishery in Mexico, with ~1600 metric tons captured every year. Ninety percent of the catch is exported live, and the export is critical for local livelihoods and quality of life. There are 500 fishermen organized into nine fishing cooperatives har-



Seafood consumption, governance, and undernourishment. (Top) Apparent per capita edible seafood consumption (2003 to 2005 average kg per year in live weight equivalent) from FAO FishStat Plus (13). Edible seafood is from fisheries and aquaculture used for human consumption. Apparent consumption is calculated for each nation by adding total seafood production to total imports and subtracting total exports. Per capita consumption divides apparent consumption by population. **(Bottom)** Governance by nation is the average of four World Bank indicators (each with a score of -2.5 to 2.5 and averaged for 2003 to 2005): rule of law, control of corruption, governmental effectiveness, and regulatory quality (11). Undernourishment categories by nation are FAO's average percentage of the population that is undernourished for 2003 to 2005 (12).

vesting the resource. Strong comanagement by cooperatives and the federal government has kept the Mexican Baja California lobster fishery from overexpanding to increase short-term export earnings at the expense of future resource availability (15).

What policy initiatives can create incentives for better governance and enhance seafood's role in food security? Developing countries rely heavily on common property resource management, in which communities organize themselves to solve the commons problem (16, 17). These institutions may fail during rapid change (e.g., new technology) or if they are not buffered from external forces (e.g., international trade) (18–20). Thus, developing countries are in a quandary with respect to seafood exports; existing common property institutions are threatened by export-oriented seafood production, and robust rights-based institutions generally require effective governance. Given the high tradability of seafood, trade policy is a natural consideration, and import tariffs theoretically can promote renewable resource sustainability (21). But seafood tariffs are likely to vio-

late World Trade Organization (WTO) rules, reduce short-term trade, and fail to differentiate among well-managed and poorly managed fisheries and aquaculture operations. In contrast, private initiatives such as ecolabeling, third-party certification, and direct sourcing have the potential to differentiate among seafood suppliers. Success of these voluntary initiatives may require that consumers are willing to pay a premium for sustainability to cover the costs of investment in sustainable governance (e.g., management), equipment (e.g., fishing gear), and infrastructure (e.g., traceability systems). Whether consumers actually will pay this premium is an open question, which suggests that other funding sources such as direct foreign aid, may be necessary. Aid providers would need to coordinate with WTO to ensure that recipients are not accused of dumping seafood on the global market.

Natural resource prices fail to reflect the cost of sustainability in many countries (22). In the short run, as producers transition toward environmental stewardship, prices rise for products like shrimp, lobster,

and salmon. But over the longer term, producers and consumers are better off because seafood supplies and livelihoods are sustainable. Price increases that reward sustainability may also raise prices of low-valued seafood, displacing fish protein from diets of the poorest of the poor in the short term. That is, when the price of the high-value product increases, demand for a substitute low-value product increases, raising its price. Research is needed to determine whether these price increases are large enough to warrant a policy intervention such as direct aid. Finally, bilateral trade between developed and developing countries highlights the importance of governance in developed countries as well. Developing countries import low-valued seafood for consumption, as well as high-valued seafood for processing, from developed countries. Sustaining these contributions to consumption and livelihood requires that developed countries also govern their resources effectively.

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Supporting Online Material

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TRENDS BY CONTINENT

Continent level of undernourishment	Percent of world population	Seafood net exports (metric tons/year)	Seafood consumption (kg/person per year)	Pop. weighted avg. governance
World				
Low	29.3	-7,838,123	21.72	0.63
Moderate	31.1	3,387,403	20.05	-0.40
High	37.9	3,182,602	9.03	-0.51
Africa				
Low	3.1	73,540	11.09	-0.13
Moderate	3.7	-935,520	10.71	-0.87
High	7.1	289,134	5.57	-0.93
Asia				
Low	6.6	-5,462,261	31.89	0.32
Moderate	22.4	3,858,470	24.21	-0.36
High	30.0	2,912,576	9.95	-0.41
Europe				
Low	11.3	-2,376,047	20.09	0.68
Moderate	0.0	0		
High	0.0	0		
North America				
Low	7.0	-2,190,357	20.54	1.17
Moderate	0.3	-51,508	9.48	-0.28
High	0.6	-11,711	5.22	-0.73
Oceania				
Low	0.4	90,891	25.69	1.79
Moderate	0.0	91,751	34.14	-0.77
High	0.0	0		
South America				
Low	0.9	2,026,111	11.07	0.07
Moderate	4.7	424,210	8.16	-0.19
High	0.1	-7,397	1.61	-0.58

Relation of exports, undernourishment, seafood consumption, and governance. Data were obtained as described in the figure legend. Low, moderate, and high refer to population-weighted averages of country-level undernourishment status. They indicate, for each continent, the proportion of the population that lives in countries where <5%, 5 to 15%, and >15%, respectively, of that country's population is undernourished. Undernourishment data are unavailable for countries representing <3% of the population of each continent, with the exception of Oceania (for which 20% of the population lives in countries without data).

CELL BIOLOGY

Propelling Progeny

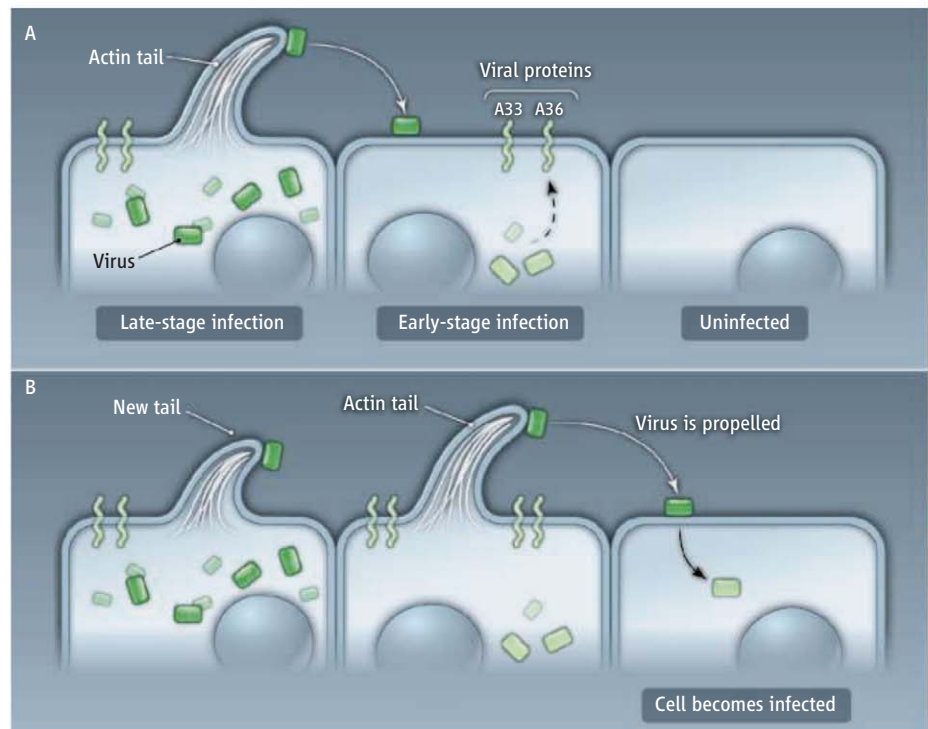
David J. Pickup

The capacity of a virus to disseminate through its host is a key determinant of virulence, but until recently, there was relatively little information on the molecular mechanisms used by viruses to accomplish this. On page 873 of this issue, Doceul *et al.* (1) propose that poxviruses spread within a host by inducing infected cells to propel progeny virions along their cell surfaces, and across those of neighboring newly infected cells, until they reach the surfaces of uninfected cells.

Poxviruses produce three types of infectious particles: the intracellular mature virion, which is released from the cell upon lysis; A-type inclusion particles, which are protein bodies that may contain up to several hundred mature virions, and which are released from the cell upon lysis; and extracellular enveloped virions, which are produced from intracellular viruses that become wrapped in two additional membranes in the host cell cytoplasm, and then released through the plasma membrane to retain one of the additional membranes. Particles of the first two types are thought to transmit virus primarily between host organisms, whereas the extracellular enveloped virions, which often make up less than 1% of total progeny, mediate most virus dissemination within a host (2). Doceul *et al.* help to explain why this extracellular form of virus is so effective at promoting virus spread.

In the simplest model of viral spread, progeny virions released from one infected cell infect neighboring cells, which in turn produce virions to infect adjacent cells. This cell-to-cell transfer may be slow, because an infected host cell must become a virus producer cell before it can transmit the virus. It may also be inefficient, because although thousands of progeny virions may be produced in a single cell, most of these may become trapped by adsorption to the small number of surrounding cells. By contrast, in the model proposed by Doceul *et al.*, based on *in vitro* experiments with infected cultured cells, the extracellular enveloped virions have a much better chance of swiftly reaching an uninfected cell. Initially, extracellular enveloped virions are propelled from the infected cell on the tips of motile microvilli, which are extended and driven forward by the actin tails (3). Vaccinia virus, the prototype

Newly infected cells use microvilli to propel extracellular enveloped virions along their surfaces, accelerating transmission to uninfected cells.



Perfect pass. (A) An extracellular enveloped virion is propelled from a productively infected cell on the tip of motile microvilli generated by actin tail formation. When the virion infects a cell, it quickly expresses the viral A33 and A36 proteins, which induce the formation of actin tail microvilli. (B) Superinfection is blocked, and the virion is passed along until it reaches the surface of an uninfected cell.

of the poxvirus family, produces two proteins (A33 and A36) that act at the host cell surface to induce the formation of such actin tails (see the figure). Although the trajectory of a virion on the tip of a microvillus is short, it can be "re-launched" if it falls back onto the infected cell surface. In this way, each virion can be passed along, above the surface of the cell, by one microvillus after another, with each cell simultaneously juggling hundreds of virions. This transmission process ends when a virus particle reaches an uninfected cell.

The suppression of superinfection (infection of a cell that is already infected) and the active surface transport of virions will keep progeny virions available to infect other cells, but this is only part of the story. Once expressed at the host cell membrane (A33 guides A36 in this localization), Abl- and Src-family kinases phosphorylate A36. This enables A36 to interact with a cascade of host cell proteins that eventually activate the actin-related proteins Arp2/3 and the process of actin polymerization (4). Because A33 and A36 are expressed within minutes of infection, the virus can quickly

enable a newly infected cell to propel extracellular enveloped virions along, thereby converting the cell into a virus distributor long before it may become a virus producer. This accelerates the transmission of virus from cell to cell.

This new distributive model fits well with known features of poxvirus dissemination. The capacities of various vaccinia virus strains to disseminate in the mouse correlate with the capacity of each virus to produce extracellular virus (5, 6). In addition, the long-range dissemination of virus is effected predominantly by cell-associated virus (7). Furthermore, vaccinia viruses can infect many types of leukocytes, but with the exception of activated T cells, most of these cells fail to support viral replication beyond the early stage (8, 9). Although this latter property appeared to rule out most leukocytes as vectors of virus spread, one interesting prediction of the new model is that leukocytes that are nonpermissive for virus replication, but capable of supporting early virus gene expression, could be extremely effective distributors of extracellular envel-

oped virus. A nonpermissive distributor cell may retain its mobility and functions better than a productively infected cell. It may also present fewer viral targets to the immune system. By contrast, extracellular enveloped virions on the surfaces of distributor cells would remain susceptible to antibody-mediated neutralization. Consistent with this prediction, the surface proteins of extracellular enveloped virions are the principal targets for antibody-mediated protection against vaccinia infections (10–12).

This mechanism of virus dissemination may be employed by other viruses, because poxviruses of several genera, including those that lack close homologs of the A36 protein, induce actin-rich projections to move virions (4). Viruses of other families, and intra-

cellular bacteria such as *Listeria monocytogenes*, also co-opt actin tails to propel them from cell to cell (although in the latter case, actin polymerization pushes a bacterium to form a protrusion at the host cell surface, which then may be internalized by another cell) (13). However, whether these microbes also possess linked mechanisms to suppress superinfection remains to be determined.

Some studies indicate that blocking virus dissemination rather than virus replication may be an effective strategy. For instance, an inhibitor of Abl-family kinases blocks actin tail formation and virus spread (14). Likewise, the compound ST-246 inhibits the formation of extracellular enveloped virions, thus halting release of the form that mediates the most dissemination within a host (15).

Therapeutics targeting such dissemination processes may have widespread potential.

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CHEMISTRY

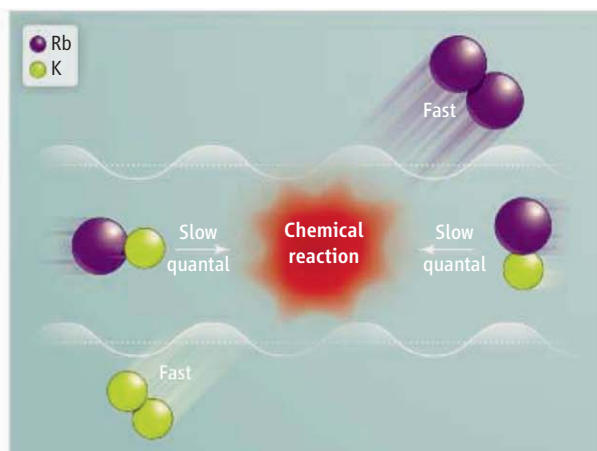
Ultracold Chemistry

Jeremy M. Hutson

Chemists usually study reactions at temperatures of tens or hundreds of kelvin, where reaction rates are averaged over many different energies and initial conditions for collision. But new techniques are now making it possible to produce molecules and trap them at temperatures within one-millionth of a degree of absolute zero. Here, all the thermal averaging is removed; the molecules occupy the lowest possible quantum translational states, and all their

motions are completely controllable. On page 853 of this issue, Ospelkaus *et al.* (1) describe chemical reactions between molecules in this new regime and find that tiny changes, such as flipping the orientation of a single nuclear spin, can have profound consequences for how (and whether) chemical reactions occur.

It has been possible to cool atomic gases and trap them at submillikelvin temperatures for almost 25 years. However, it has only recently become possible to produce molecules in this “ultracold” regime. A major breakthrough came in 2003, when several groups (2–5) succeeded in producing molecules in atomic



gases by magnetoassociation, in which pairs of atoms are coherently converted into molecules by carefully controlled magnetic field ramps. However, the molecules are extremely weakly bound, by only about 1 part in 10^8 of normal chemical bond energies. Coherent laser techniques were then developed to transfer these ultracold molecules to deeply bound states that can be regarded as chemically bonded. This has been achieved for only three species: singlet KRb (6) and Cs_2 (7), and triplet Rb_2 (8).

Ospelkaus *et al.* describe pairs of ultracold KRb molecules that collide and exchange atoms to form $\text{K}_2 + \text{Rb}_2$ (see the figure). They used ^{40}K and ^{87}Rb to form KRb molecules that are fermions, with half-integer total spin. As two identical fermions avoid one another

Cooling atoms and molecules to ultralow temperatures allows the study of quantum-controlled chemical reactions.

A cold reaction. Two KRb molecules at temperatures below $1\ \mu\text{K}$ can react to form $\text{K}_2 + \text{Rb}_2$, but the rate is suppressed by fermion symmetry (the Pauli principle) when the two molecules are in identical quantum states.

because of the Pauli principle, the collision rate at low temperature is reduced and the KRb molecules can survive for several seconds as long as they are all in the same internal quantum state. However, if some molecules are converted into a different internal state by flipping the orientation of the nuclear spins, collisions are no longer suppressed and the molecules react 10 to 100 times as fast.

Ultracold molecules have many potential applications. Dipolar molecules are expected to form a variety of new quantum phases (9). They may be used as qubits in quantum computers (10) or to probe fundamental physical symmetries through measurements of the electric dipole of the electron (11). For these applications, chemical reactions provide a trap loss mechanism that must be avoided. Fortunately, there are several routes by which this may be achieved. There are some alkali metal pairs, such as RbCs, for which atom exchange reactions are energetically forbidden, and even where such reactions are allowed, they may be prevented by placing the molecules in an optical lattice (7) or by creating repulsive interactions between molecules with electric fields (12).

Ospelkaus *et al.* have also studied the reactions of KRb with ultracold K and Rb atoms. With K atoms, there is a fast reaction to form $K_2 + Rb$, which releases kinetic energy and ejects the products from the trap. With Rb, however, the reaction to form $Rb_2 + K$ is energetically forbidden. Provided both the Rb atoms and the KRb molecules are in their absolute ground states, the molecules are long-lived. However, if either the atoms or the molecules are placed in a different nuclear spin state, fast loss is again observed. This is something of a puzzle, as there is still no chemical pathway available and collisions that change only the orientation of a nuclear spin should be suppressed by centrifugal barriers at these temperatures.

Magnetoassociation is not the only approach being used to form ultracold molecules. Deeply bound ultracold molecules have also been produced from atoms by photoassociation, in which laser light is used to transfer

pairs of atoms directly into molecular states by spectroscopic transitions (13). However, the yields of ground-state molecules have been relatively low and quantum mechanical coherence has not been achieved.

Both magnetoassociation and photoassociation are limited to molecules formed from atoms that can themselves be laser-cooled. In practice this limits them to molecules formed from alkali metals, alkaline earths, and a very few other atoms. Such molecules have a limited chemistry, so there is an intense parallel effort to cool preexisting molecules to the ultracold regime, using techniques such as buffer-gas cooling in cryogenic helium (14) and deceleration of molecular beams with switched electric and magnetic fields (15). These methods can be applied to a wider range of molecules, including chemically important polar molecules such as ammonia and free radicals such as OH and NH. These “direct cooling” methods have only achieved

temperatures of 10 to 100 mK, which are not quite in the ultracold regime. Nevertheless, it is likely that ways will soon be found to cool these species to submillikelvin temperatures as well, paving the way for a new type of quantum-controlled ultracold chemistry.

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CHEMISTRY

CO Prefers the Aisle Seat

Michael S. Altman

Atomic steps are common defects at surfaces that can play a role in many physical and chemical phenomena. This simple idea is one reason why small metal particles, which can expose many low-coordination catalytically active sites at steps (see the figure), are used in commercial catalysts. On page 850 of this issue, Tao *et al.* (1) show that a common molecular reactant can reversibly alter the density and arrangement of active step edges under realistic reaction conditions.

A basic understanding of catalysis is often sought through the study of stepped, macroscopic single crystal surfaces as model systems, produced by miscutting a bulk crystal precisely away from a low-index plane. The term “vicinal” is used to describe these surfaces. Surface science experiments are traditionally carried out in a controlled ultrahigh vacuum (UHV) environment (10^{-10} to 10^{-6} torr), partly because of pressure limitations of characterization techniques that often use electron beams. This approach allows systematic empirical study, but does not access the conditions at or near atmospheric pressure under which real catalysts must operate.

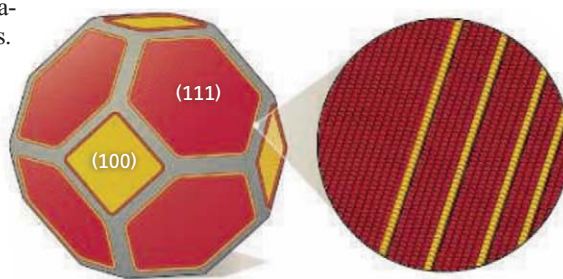
Tao *et al.* have bridged this “pressure gap” with state-of-the-art ambient pressure x-ray photoelectron spectroscopy and scanning tunneling microscopy techniques developed in their laboratory. They have used these techniques to study carbon monoxide (CO) adsorption on vicinal platinum (Pt) (111) surfaces at pressures up to 0.1 to 1 torr (1 atm = 760 torr) at room temperature. CO is a reactant in many important industrial catalytic processes. The results reveal that, at low CO pressures ($\sim 10^{-7}$ torr), CO adsorption drives step rearrangement that culminates in the formation of double-height atomic steps. Under these conditions, the CO saturation coverage on a Pt (111) surface remains below one monolayer at room temperature as a result of strong mutual CO repulsion (one monolayer = one CO molecule per surface Pt atom). Increasing the gas pres-

sure forces more CO onto the Pt surface, until a coverage of one monolayer is reached under near-ambient conditions.

The authors observe an additional transformation of the step morphology at ambient conditions: Platinum nanoclusters just a few atoms in size form at step edges. This transformation appears to be linked to enhanced CO occupation of the step edge sites. It occurs despite the energy price that must be paid to increase the number of undercoordinated sites along the now effectively longer steps. The nanoclusters disappear again when the CO pressure is reduced.

The results of density functional calculations (1) suggest that the highly compressed CO layer relaxes by tilting with respect to the surface normal direction toward the down-step direction. CO molecules at the step edge enjoy the greatest relaxation. The formation of nanoclusters at the Pt step edges allows a higher concentration of CO to occupy low-coor-

Don't forget the steps. A close-up view of the surface of a Pt crystalline particle shows low-index facets and atomic steps (orange) in the vicinal region bordering a facet. Tao *et al.* show how the catalytically active step edges transform under the influence of a common reactant.



Department of Physics, Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong S.A.R., China. E-mail: phaltman@ust.hk

dination sites, thus enabling a greater overall system relaxation. This effect is analogous to step proliferation, a well-known stress relief mechanism in strained heteroepitaxial films on surfaces (2). The relaxation achieved by CO tilting at steps can be likened to the reason why some of us have a preference for the aisle seat. The advantage that CO has is its ability to force a rearrangement of seats that allows more molecules to reside at the coveted aisle.

The initial stage of the surface rearrangement reported by Tao *et al.* is similar to the oxygen-induced step doubling observed previously on vicinal surfaces of Pt and other metals in UHV (3–7). If this behavior is indeed general, then we may expect to find CO-induced nanocluster formation at step edges of many vicinal metal surfaces under ambient conditions.

The results of Tao *et al.* help to bridge the gap between UHV studies of step morphology and dynamic catalytic particle changes under ambient conditions (8, 9). A crystalline particle changes its shape by mass transport over its surface. Because steps are the mass sources and sinks for this process, shape changes must involve step motion. Experiments providing correlated chemical and morphological information, such as those of Tao *et al.*, can elucidate the atomic mechanisms that lead to particle shape changes.

Changes in step morphology that may occur as catalytic reactions progress must now be considered in studies of catalytic behavior. However, better understanding of catalyst behavior requires knowledge not only of the structures—as provided by Tao *et al.*—but also the kinetics of nanocluster formation. Entropy also reduces step free energies on

clean surfaces, resulting in equilibrium fluctuations of step shape that become more pronounced as temperature is increased (10). To explore the possible impact of these effects on catalysis, the methods used by Tao *et al.* should be extended to even higher pressures and temperatures.

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CLIMATE CHANGE

Ice Age Rhythms

R. Lawrence Edwards

What caused the series of more than 20 ice ages that have come and gone during the past 2 million years of Earth history? On page 860 of this issue, Dorale *et al.* (1) reveal a new twist in the most recent ice age cycle and demonstrate once again the rapidity with which large ice sheets can come and go.

The great Northern Hemisphere ice sheets of past ice ages have sculpted the surfaces of much of the northern continents and shifted positions of shorelines worldwide (see the figure). The changing climates of the ice ages provided the environmental backdrop for the last episode of human evolution. Advancing and retreating shorelines modified migration routes for humans and other species. Some of today's most productive soils in the American Midwest, central China, and southeastern Europe developed on wind-blown silt (loess) deposited during glacial times. The waxing and waning of ice sheets thus shaped much of today's world, and knowledge of their causes may help us understand some of the challenges that we face in the coming decades and centuries of climate change (2).

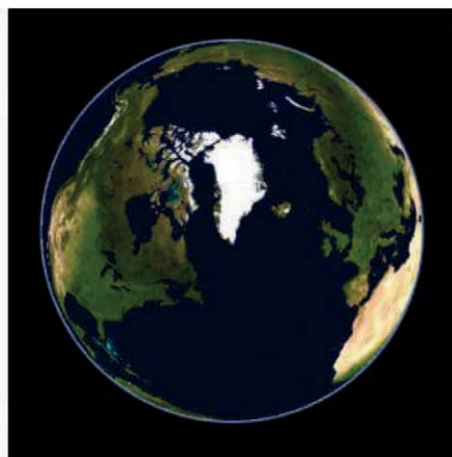
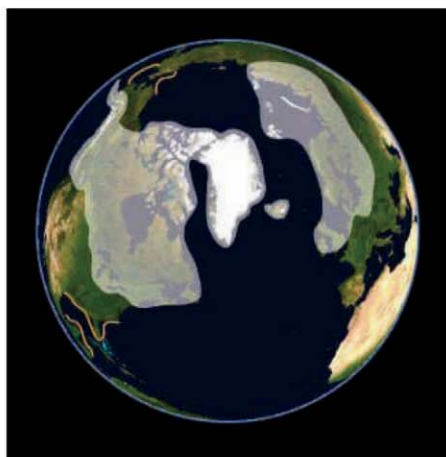
Given that climate differs widely from region to region, reconstructing hundreds of thousands of years of climate history would

seem to be an impossible task. Earth scientists have therefore sought measuring sticks that integrate climate. One such measure is sea level, an inverse measure of the volume of ice stored above sea level on continents. As the continental ice sheets grew and decayed, sea level dropped and rose, falling to as low as ~130 m below present levels and rising as high as several meters or more above present levels. Sea level is thus a good (but not perfect) measure of the waxing and waning of the ice sheets.

Evidence for high sea level ~81,000 years ago provides new insight into ice age history.

For this reason, accurate sea-level reconstruction is a central goal of climate research. The gross features of the curve are now known (3), but there is still a need to improve resolution, accuracy, and precision, both in time and elevation, and to resolve discrepancies. Seemingly small errors can have major implications: An error of 6 m in sea-level elevation is equivalent to the presence or absence of all the ice currently on Greenland.

Early work on sea-level history (4, 5) found a strong link between sea level and the inten-



Whither the 100,000-year cycle? Previous data suggested that each of the last several ice age cycles took ~100,000 years. If this were the case, then ~81,000 years ago, Earth's ice cover should have been intermediate between the glacial (14) and the modern state. Dorale *et al.* present evidence that instead, ~81,000 years ago, ice cover was similar to today's. Orange lines indicate shoreline positions in the ice age world. Sea ice is not depicted.

Department of Geology and Geophysics, University of Minnesota, Minneapolis, MN 55455, USA. E-mail: edwar001@umn.edu

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sity of Northern Hemisphere summer sunlight (insolation), the latter calculated from known changes in the geometry of Earth's orbit and rotation axis. This observation provided support for the Milankovitch (6) or astronomical theory of the ice age cycles. However, the early data also posed a serious problem. Calculated sunlight variations have periods of ~23,000, ~41,000, and ~100,000 years, with the latter periodicity much weaker than the others. The ice age cycles contain the same periodicities, but for the last several cycles, the dominant period is ~100,000 years, the least significant in the sunlight calculation (4). A generation of scientists has strived to explain this "100,000-year problem" through nonlinear responses of Earth's climate to changes in the seasonal distribution of sunlight (7, 8) or to processes not related to the sunlight calculation at all.

Dorale *et al.* provide evidence for high sea level at ~81,000 years ago, in the middle of the most recent 100,000-year cycle. This result challenges the observational basis for much of the discussion over recent decades. A high sea level at ~81,000 years ago is not consistent with a 100,000-year beat, but it does coincide with calculated high Northern Hemisphere summer sunlight, and thus supports a simple version of the Milankovitch theory. If verified, this sea-level high may be considered an exception to the 100,000-year cycle, in which high summer sunlight caused the ice sheets to melt—an exception with precedent, given evidence for another off-beat event ~229,000 years ago (7).

Dorale *et al.* dated layers of the mineral calcite, which were deposited like bathtub rings from pools of water in Mallorca caves, in the western Mediterranean. Because the pools are connected to the sea through underground passages, the layers record sea level at the time they formed. Using this approach, Dorale *et al.* inferred sea levels similar to modern values ~81,000 years ago. They estimated maximum rates of sea level rise of ~2 m per century. This rate is high, but not unprecedented in the geologic record. It exceeds by several times those predicted for the next century (9).

Others will likely test Dorale *et al.*'s inference of low ice volume 81,000 years ago. A major question relates to the flow and bend of the solid Earth, such that sea level is not solely dependent on ice volume. Earth's shape, mass distribution, and gravitational field change continually in response to the redistribution of water between the ice sheets and oceans during the ice age cycles (10, 11). Because of the high viscosity of Earth's mantle, the solid Earth responds slowly (over thousands of years) to the rapid redistribution of water and ice on the surface. The physics of this process is well known, but the calculation requires knowl-

edge of the elastic properties of the lithosphere (Earth's rigid outer shell), the viscosity structure of the mantle and the history of ice and water distribution on Earth's surface, which are difficult to quantify. Nevertheless, the process has been modeled for different times in the ice age cycle (10, 11). Dorale *et al.*'s findings should spur further studies, with an eye toward the Mallorca region.

A number of previous studies have estimated sea level ~81,000 years ago. Some of these estimates appear to agree (12) with Dorale *et al.*'s findings, whereas others appear to disagree (13). One problem with comparing these studies is the possibility—and in some cases probability—that discrepant sea-level elevations may represent different sea levels at different times, given plausible dating errors. Future studies that determine sea levels at different times at the same place may help to resolve the discrepancies. Regardless of the ultimate verdict on sea level ~81,000 years ago, Dorale *et al.*'s findings will stimulate ideas, discussion, and new studies of ice age history and causes.

GENETICS

Genetic Control of Hotspots

Vivian G. Cheung,¹ Stephanie L. Sherman,² Eleanor Feingold³

Both chromatin and DNA sequence account for individual differences in the location and frequency of genetic recombination.

With the exception of identical twins, individuals have different genetic makeup, which results from two key processes. During meiosis, maternal and paternal homologous chromosomes assort randomly to form daughter cells (gametes), thus generating different combinations of maternal and paternal chromosomes. Additional variation is generated by recombinations or crossovers, in which parts of homologous chromosomes are exchanged, resulting in a new combination of parental alleles. On pages 835, 836, and 876 of this issue, Parvanov *et al.* (1), Baudat *et al.* (2), and Myers *et al.* (3) report the identification of a mammalian gene—PR domain containing 9 (*PRDM9*)—that controls the extent to which crossovers occur in preferred chromosomal locations,

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known as "hotspots" (see the figure).

In addition to contributing to genetic variation, recombination is critical to the success of meiosis. A physical bridge that is built around the point of exchange—the chiasma—ensures correct assortment of chromosomes into gametes. The location of a chiasma is important because an exchange that occurs too close to the telomere or centromere of a chromosome can confer instability and lead to abnormal chromosome segregation (4). In humans, this type of error is startlingly common. Aneuploidy [either monosomy (only a single copy of a chromosome, rather than a pair) or trisomy (three copies of a chromosome)] is estimated to occur in 10 to 25% of all conceptions and is the leading cause of pregnancy loss as well as developmental disabilities (5).

Even though meiosis and meiotic recombination are fundamental cellular processes, the genes and mechanisms involved are poorly understood. In mouse recombination hotspots, histone proteins are often modified by methylation or hyperacetylation (6),

¹Howard Hughes Medical Institute, Departments of Pediatrics and Genetics, University of Pennsylvania, Philadelphia, PA 19104, USA. ²Department of Human Genetics, Emory University, Atlanta, GA 30322, USA. ³Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA 15261, USA. E-mail: vcheung@mail.med.upenn.edu

and in many human hotspots, a degenerate DNA sequence of 13 nucleotides (a 13-mer) is found (7). These findings have generated debate over whether individual variability in recombination rates and locations of cross-overs is explained by DNA sequence signatures or epigenetic marks.

PRDM9 is a zinc finger protein that acts as a histone methyltransferase that trimethylates lysine 4 of histone 3 (H3K4me3). The *PRDM9* gene is highly polymorphic, especially in its zinc finger arrays that bind DNA. The polymorphic forms of PRDM9 recognize different DNA sequences and therefore can promote crossovers at different chromosomal sites among individuals. The finding that a polymorphic chromatin modifier binds to different DNA sequences is pivotal, because

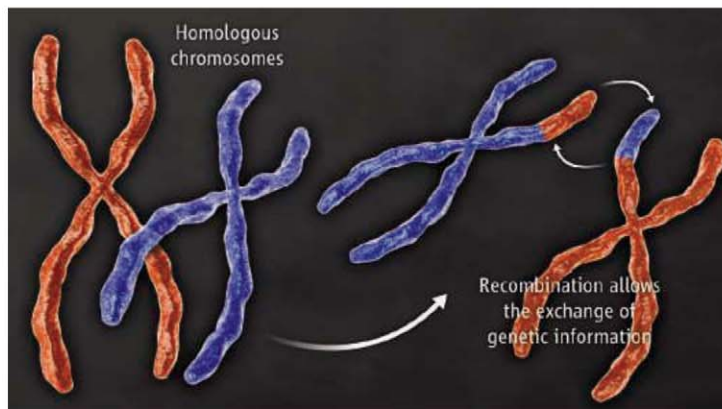
Genetic variation by crossover. Recombination events (crossovers) occur at different sites along chromosomes. Some sites, so-called “hotspots,” are used more often than other sites.

it suggests that both chromatin and DNA sequences are important in meiotic recombination.

One elegant feature of the three studies is that *PRDM9* was identified through mouse and human studies and by computational analyses. The mouse and human work treated the numbers of crossovers as quantitative phenotypes in genetic analyses. In the mice, linkage scans were used to identify the chromosomal regions that segregate with high and low recombination rates in known hotspots. Parvanov *et al.* used a mouse cross to fine-map the candidate regions to four genes, and argued that of these, *PRDM9* is the only logical candidate gene. Similarly, Baudat *et al.* narrowed a region that they mapped previously (8) and identified *PRDM9* as the candidate gene. They then took the study directly into a human population of Hutterites and found three allelic forms of *PRDM9*: A, B, and I on human chromosome 5. The A form was the most common and accounted for 94% of alleles. They further noted that the I allele encodes a protein that does not recognize the 13-mer hotspot motif. The authors show that usage of hotspots by heterozygous individuals who carry one copy of A and a copy of I or B forms of PRDM9 is significantly different from that of individuals with two copies of the A form of PRDM9. The variation in the zinc finger array alone accounts for 18% of phenotypic variation. Myers *et al.* used a computational approach

and found that human PRDM9 is the only zinc finger protein that binds to the 13-mer motif. They also demonstrated that the 13-mer motif does not appear to be active in chimpanzees. By cross-species comparisons, they suggest that the difference may be attributed to rapid evolution in humans rather than loss in chimpanzees.

An important lesson from this discovery is that results from genetic studies can direct mechanistic analyses. Often, variation is an unwanted complication in mechanistic studies; here the authors took advantage of the variation in recombination events to identify



PRDM9 as the genetic determinant and paved the way for examining the role of chromatin in recombination. Analyses of yeast and mouse hotspots have shown that H3K4me3 markers are enriched in recombination initiation sites (6, 9). Results from Myers *et al.*, Baudat *et al.*, and Parvanov *et al.* further support the role of chromatin modifiers. Despite using fairly crude phenotypes (for humans, the phenotype was the percentage of recombinations in hotspots), these investigators were able to identify a single gene, thus suggesting that variation in recombination might be regulated by a few genes with large effects. Identifying additional genes will refine future mechanistic studies.

What are the interacting partners of PRDM9 and how do they work together to direct recombination? Not all the sites in the human genome with the 13-mer motif have a crossover, so it is unclear how PRDM9 decides which sites to use. Additionally, because *PRDM9* explains only an estimated 18% of individual variation in hotspot usage, other genes must contribute to this variation. If more fine-scale phenotypes such as specific types (e.g., those with the 13-mer motif) of recombination are used, it may be possible to remove some measurement noise and therefore have greater power to detect genetic association. Previously, other

human genes including *RNF212* were identified as genetic determinants of the number of recombinations per meiosis (10, 11); perhaps there are interactions between these genes and *PRDM9*.

At the interface between mechanisms and disease, an important question is whether there is a minimum number of recombinations required for proper meiosis. In yeast, when crossover events were diminished by deletion of the histone methyltransferase Set1, new crossovers appeared in trimethylation “deserts” (9). This and other studies (12, 13) suggest a minimum number of required recombinations for proper meiosis; if so, what genes or pathways monitor this process? If this global monitoring system (which likely includes PRDM9) fails, aneuploidy may result. If this is the case, an enrichment of certain *PRDM9* variants would be expected among women with recurrent miscarriages and infertility and those who have had aneuploid pregnancies. The finding of the three studies opens the door to understanding the balance of successful gamete formation and maintenance of genetic diversity. Ultimately these studies will have clinical impact beyond basic science, as they will inform us about how errors of recombination lead to infertility, miscarriages, and developmental disabilities.

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MICROBIOLOGY

Feasting on Minerals

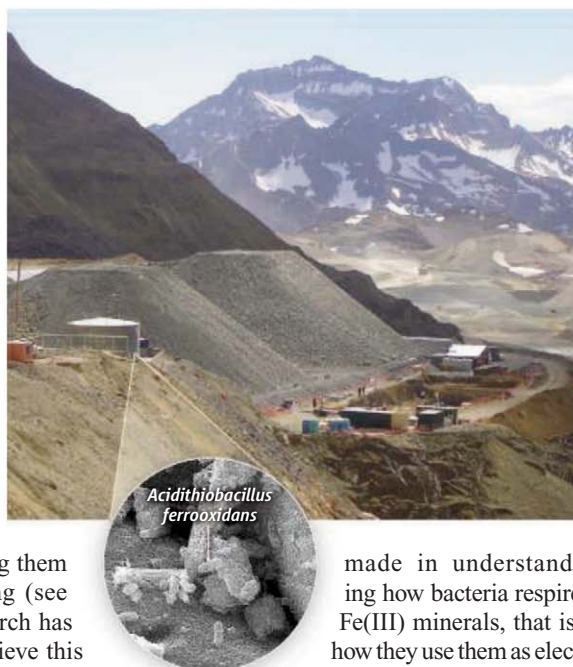
Dianne K. Newman

Far up in the Chilean Andes, in remote arid regions seemingly inhospitable to life, intrepid microorganisms thrive on a diet of rocks and air. Unfazed by long periods of desiccation or high ultraviolet energy flux, they grow in baths of sulfuric acid replete with toxic metals. The microbes fix carbon dioxide into biomass by exploiting the energy to be gained by “eating” (oxidizing) minerals that contain reduced forms of iron and sulfur, such as chalcopyrite (CuFeS_2). Through their metabolism, these microbes mobilize precious metals from ore deposits into solution, making them powerful catalysts for biomining (see the first figure) (1). Recent research has begun to elucidate how they achieve this remarkable feat.

The Atacama Desert is one of the most extreme environments on Earth where one can find mineral-eating microorganisms, but it is far from the only place where they live. Bacteria and archaea that grow by oxidizing ferrous iron, Fe(II), have been found in habitats ranging from the deep sea (2) and acid mines (3) to wetlands (4), groundwater (5), and lakes (6). Their metabolic activities can alter the geochemistry of their surroundings, influencing the weathering of minerals and the cycling of major and minor elements.

Some mineral-eating organisms catalyze mineral oxidation by harnessing energy from the Sun, others perform this feat in the dark; some only thrive at pH ~ 1 , others at pH near 7; some require oxygen for their metabolism, others subsist strictly in its absence (7). But they all produce ferric iron, Fe(III), as a metabolic waste product, which, in many cases, rapidly precipitates and encrusts the cells. Other bacteria use these Fe(III) mineral products as terminal electron acceptors for respiration, like humans use oxygen. Yet for the cells that produce them, they pose something of a gastronomic hazard—the equivalent of choking on one’s own meal.

In the past decade, much progress has been



made in understanding how bacteria respire Fe(III) minerals, that is, how they use them as electron acceptors (8), but much less is understood at the molecular level about how bacteria eat Fe(II) minerals, that is, how they use them as electron donors. Mineral-eating bacteria are challenging to cultivate and manipulate in the laboratory because of their unusual growth constraints, such as extremely acidic pH or a requirement for trace amounts of oxygen (9). However, it can be done, and recent studies have begun to shed light on their biochemistry.

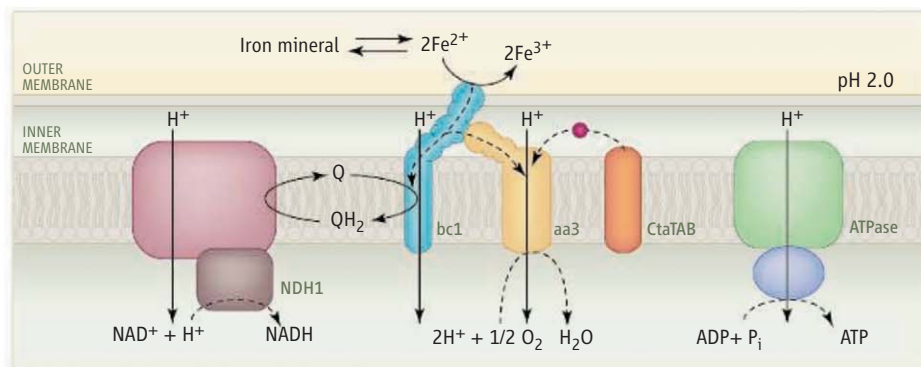
Most of what we know comes from bio-

Molecular studies are elucidating how microbes can eat iron-containing rocks, but many questions remain.

Unusual diet. The bioleaching pile from the Andina copper mine in Saladillo, Chile, contains $\sim 60,000$ tons of ore (mainly chalcopyrite). Microorganism such as *Acidithiobacillus ferrooxidans* inhabit these piles and catalyze the leaching of precious metals. The scanning electron micrograph (inset) shows the cells, 1 to 2 μm in length, attached to a chalcopyrite particle.

chemical studies of the acidophile *Acidithiobacillus ferrooxidans* (see the first figure, inset) (10), first described almost 60 years ago (11). More recently, molecular experiments with various model Fe(II)-oxidizing microorganisms (12–16) have identified specific electron carriers such as c-type cytochromes (proteins that covalently coordinate heme groups) that appear to be responsible for delivering electrons from Fe(II) to the respiratory chain. These studies have led to a cartoon-level understanding of the bioenergetic pathways in this process (see the second figure) (10), but many details remain obscure.

For example, how do mineral eaters control their diets? The oxidation substrate, Fe(II), can be a potent toxin in the presence of oxygen, generating reactive oxygen species. But even under anaerobic conditions, Fe(II) can be toxic for certain species. The molecular basis of this toxicity is unknown, but at least one bacterium appears to use Fe(II) oxidation to detoxify rather than to grow (17). Furthermore, all Fe(II) oxidizers require trace quantities of iron to support the biosynthesis of metabolic cofactors. How do they



Electron transfer in *Acidithiobacillus ferrooxidans*. Fe(II) is oxidized to Fe(III) by proteins on the cell surface. These proteins transfer electrons from Fe(II) to other parts of the respiratory chain, leading to the generation of an energized membrane in the cell. This energy is used to generate metabolites necessary for growth and other cellular functions. ATP, adenosine 5'-triphosphate; ADP, adenosine 5'-diphosphate; NADH, nicotinamide adenine dinucleotide; NADPH, NAD phosphate; NDH1, NADPH dehydrogenase; bc1 complex, ubiquinol-cytochrome c reductase; aa3, cytochrome oxidase; CtaTAB, likely involved in heme synthesis and export to cytochrome oxidase; ATPase, an enzyme that catalyzes the decomposition of ATP into ADP and phosphate.

juggle this demand with that for much larger amounts of iron for energy generation? And how do they transport Fe(II) into and Fe(III) out of the cell? Putative iron transporters have been identified (12), but little is known about how they work.

It is becoming clear, however, that mineral eaters have a range of gastronomic strategies. Acidophiles circumvent the iron transport issue (at least for growth purposes) by displaying their Fe(II)-oxidizing enzymes on their surface (10) and catalyzing Fe(III) mineral formation on organic fibrils that extend away from the cell (18). In contrast, some neutrophilic Fe(II) oxidizers appear to localize their Fe(II)-oxidizing machinery inside their cells (12, 13); in some cases, Fe(III) oxides form in the cells (19), in others outside (20). What explains the difference?

Microorganisms have a dazzling command over their intracellular organization (21), so the localization of their metabolic machinery cannot be random. It may be that the way iron presents itself to the cell varies according to the niche different mineral-eaters occupy. Furthermore, mineral-eating organisms differ in the complexity of their internal membrane structures, which may affect their strategy for electron transfer. Another factor

will be whether an organism can produce Fe(III)-binding molecules or polymers to prevent iron from precipitating internally.

The electron transport machinery in mineral-eating organisms also deserves attention. c-type cytochromes appear to be important players in the electron transport chains of all mineral-eating organisms, but they differ widely in size and composition. What is the extent of their diversity? Why do some organisms use Fe(II) oxidases with 10 hemes, whereas others require far fewer? How do these enzymes compare to those of their respiring counterparts that convert Fe(III) to Fe(II)? How are they distributed in the cell?

The more we know about how microbes eat minerals, the better we will understand the roles these organisms have played and still play in shaping the geochemistry of many environments on Earth. These insights will also help in harnessing their remarkable metabolisms for industrial applications.

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CHEMISTRY

Radical Ligands Confer Nobility on Base-Metal Catalysts

Paul J. Chirik¹ and Karl Wieghardt²

The industrial preparation of many chemicals relies on the unparalleled rate and selectivity enhancements offered by metal compounds in solution. In many cases, the best catalysts rely on the scarcest elements, such as rhodium, iridium, and platinum. The cost of these materials has long driven efforts to make soluble catalysts out of cheaper, more Earth-abundant metals (1), often by modifying their reactivity with their surrounding ligands. This is especially true for catalyzing reduction-oxidation, or redox, reactions, which are critical not only in catalysis but in energy generation and storage. Such reactions usually change the oxida-

tion state of the metal in solution. We discuss why there can be advantages to having the redox changes occur in the ligands instead.

One major obstacle in replacing noble metals with more common ones stems from the differences in electronic structure. A noble metal like platinum often favors two-electron redox changes to promote bond-making and breaking events. For the base metals, one-electron redox changes occur more frequently and present challenges for controlling reactivity and stabilizing or maintaining the function of the catalyst.

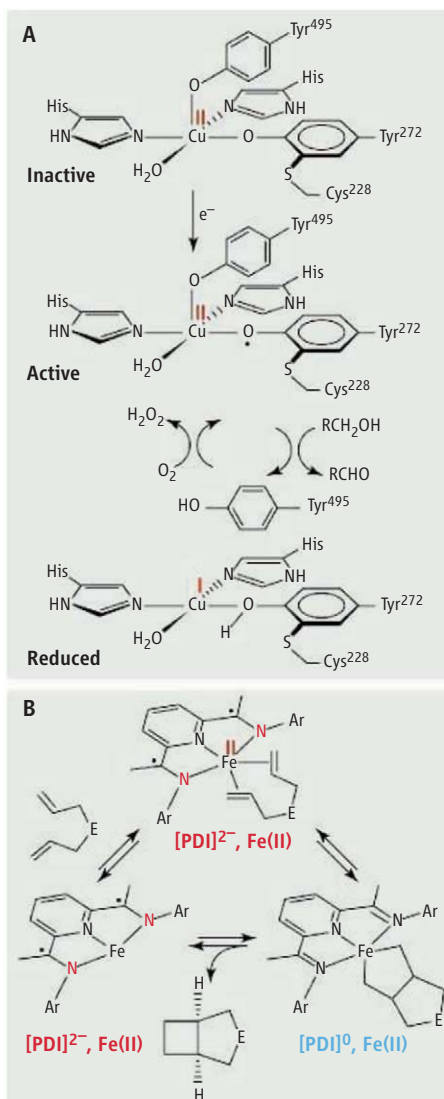
To mimic noble metals, one-electron redox changes must be suppressed and two-electron redox events facilitated. Most ligands used in inorganic chemistry, such as ammonia or triphenylphosphine, are not “redox-active”—the energy needed to oxidize or reduce them by even one electron is much greater than

The oxidation state of metals such as copper and iron can be stabilized by organic ligands that add or lose electrons and facilitate catalysis.

that needed to change the oxidation state of the metal, so changes in electronic structure occur at the metal. Redox-active, or “non-innocent,” ligands (2, 3) have more energetically accessible levels that allow redox reactions to change their charge state. For example, NO may bind as a cation in a linear geometry or an anion with a bent geometry.

Redox-active ligands have long been recognized in coordination chemistry. Gray and co-workers determined that square-planar cobalt (4) and nickel (5) dithiolene complexes were best described as metal(II) compounds with two ligand radical anions, rather than the metal in the +4 oxidation state and –2 ligands. Catecholates and diimines also have a distinctive ability to form radical species, which normally would be unstable in solution, when they are bound to metal centers. The extended network of π bonds in these ligands allow

¹Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY 14853, USA. ²Max-Planck Institute for Bioinorganic Chemistry, Stiftstrasse 34–36, D-45470 Mülheim an der Ruhr, Germany. E-mail: pc92@cornell.edu; wieghardt@mpi-muelheim.mpg.de



them not only to stabilize radical species but also to facilitate reversible reactions with the metal center that may involve radical formation. Spectroscopic, structural, and magnetic data, theoretical modeling, and patterns of reactivity are often needed to assign the true electronic structure description of a transition metal complex (6).

The use of metal complexes with radicals on the supporting ligands as catalysts also draws inspiration from enzymatic reactions of certain metalloproteins (7). One of the best understood examples is galactose oxidase, which performs the two-electron oxidation of alcohols to aldehydes. A Cu(II) ion is coordinated to a modified tyrosyl radical, and this intricate bonding situation gives rise to the function of the enzyme (8) (see the figure, panel A). This phenomenon may be pervasive in metal-containing redox proteins. However, it may be difficult to detect when two radical ligands are present, because they may strongly couple through a central metal

ion and not produce a distinctive signature in spectroscopic studies (9).

For synthetic iron catalysis, the bis(imino)pyridine family of ligands, pioneered in base-metal olefin polymerization catalysis by Brookhart and co-workers (10) and Gibson and co-workers (11), can coax the metal into the appropriate electronic configuration to engage in chemistry equal or superior to reactions catalyzed by precious metals. The ligand is stable in four chemically accessible oxidation levels (neutral, as well as mono-, di-, or trianions). The mono- and trianions are π radicals—they have an odd electron and a spin state of 1/2—whereas the two electrons of the dianion may spin-pair to form a singlet ground state or stay unpaired and form a triplet ground state (12, 13).

Many catalysts perform just one type of reaction, but the iron complex $(iPrPDI)Fe(N_2)_2$ can be used in a number of reactions, such as the hydrogenation and hydrosilylation of olefins (14), as well as the cyclization of enynes and diynes (15) (see the figure, panel B; iPr is isopropyl, and PDI is a pyridinediimine ligand). A combination of spectroscopic techniques and density functional theory calculations established that this formally iron(0) compound (with a neutral ligand) has the physical oxidation state of an intermediate spin iron(II) compound, where the metal has transferred two electrons to the $iPrPDI$ ligand (16, 17).

We illustrate the role of the ligand in reactions catalyzed by $(iPrPDI)Fe(N_2)_2$ with a cyclization reaction. A saturated two-ring compound forms from an organic molecule with double bonds at each end—this $[2\pi + 2\pi]$ cycloisomerization of α, ω -dienes forms bicycloheptanes (18). Because of the constraints of orbital symmetry, examples of this reaction outside of photochemistry are rare. However, under mild, room-temperature conditions, the active catalyst, $(iPrPDI)Fe$, formed when the N_2 ligands are displaced by substrate promotes efficient and complete cyclization. Model complexes have also been prepared to probe the electronic structure of likely intermediates formed during the catalytic cycle. Spectroscopic and crystallographic studies support a pathway where the iron(II) oxidation state is

maintained throughout the catalytic cycle.

Although the overall reaction is a formal reduction (the C=C double bonds are converted to single bonds), no oxidation state changes occur at the metal. The required electrons to form the C—C single bonds are supplied by the ligand. Similarly, the reductive elimination of the product does not form Fe(0) but reduces the ligand again. By confining all redox changes to the ligand, we believe energetic balance is maintained between intermediates in the cycle, and true iron(0) (with a d^8 electron configuration) species are avoided. Such compounds are often detrimental in reduction catalysis because they often decompose by ligand loss, and will result in deposition of the metal.

Redox-active metal-ligand combinations, once the domain of coordination chemists and spectroscopists interested in structures, are undergoing a rebirth and entering the realm of catalysis (19). Performing metal-mediated redox chemistry where oxidation state changes occur at the ligand while the metal's electronic configuration is maintained is a much broader concept that is likely to inspire new transformations (20) and, ultimately, new applications.

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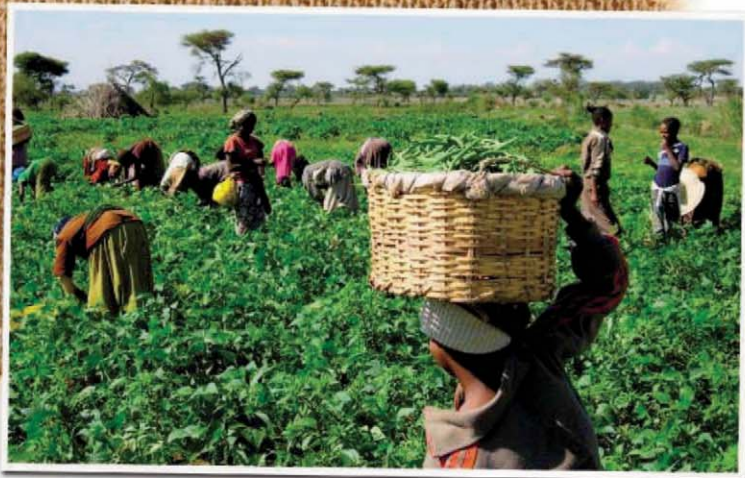


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INTRODUCTION

Feeding the Future

FEEDING THE 9 BILLION PEOPLE EXPECTED TO INHABIT OUR PLANET BY 2050 WILL BE an unprecedented challenge. This special issue examines the obstacles to achieving global food security and some promising solutions. News articles take us into the fields, introducing farmers and researchers who are finding ways to boost harvests, especially in the developing world. Reviews, Perspectives, and an audio interview done by a high school intern provide a broader context for the causes and effects of food insecurity and point to paths to ending hunger.

We have little time to waste. Godfray *et al.* (p. 812) note that we have perhaps 40 years to radically transform agriculture, work out how to grow more food without exacerbating environmental problems, and simultaneously cope with climate change. Although estimates of food insecurity vary (Barrett *et al.*, p. 825), the number of undernourished people already exceeds 1 billion; feeding this many people requires more than incremental changes (Federoff *et al.*, p. 833).

Scientists and engineers can make a big difference at every step from field to fork, from providing new strategies to smallholder farmers who must balance the needs of livestock and crops (Herrero *et al.*, p. 822) to helping farmers get the most from fertilizers, water (Vince, p. 800), soil (Hvistendahl, p. 801), and seeds (Tester and Langridge, p. 818; Pennisi, p. 802). Innovation will be key to monitoring all stages of food production (Gebbers and Adamchuk, p. 828), from defending harvests against pests and disease (Pennisi, p. 804; Normile, pp. 806 and 807) to providing critical information and infrastructure (Stone, p. 808). And training enough scientists in all these areas will be essential (see associated *Science Careers* profiles at www.sciencereers.org).

As Vince's profile (p. 798) of one Ugandan farmer illustrates, science and technology alone cannot guarantee food security. Economic, political, and psychological issues also play key roles. Yet there is optimism that a Green Revolution is possible in Africa (Ejeta, p. 831), although maintaining good governance throughout the world is crucial to success (see the associated Policy Forum by Smith *et al.*, p. 784).

Much of this special issue focuses on how to increase the supply of basic staples. But Stokstad (p. 810) examines one idea for reducing demand: eating less meat; and Vogel (p. 811) highlights an alternative source of protein: insects. These alternatives are possibly unappetizing to many, but the quest for food security may require us all to reconsider our eating habits, particularly in view of the energy consumption and environmental costs that sustain those habits. As this special issue shows, science can help to make the choices less unpalatable.

—CAROLINE ASH, BARBARA R. JASNY, DAVID A. MALAKOFF, ANDREW M. SUGDEN

Food Security

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NEWS

From One Farmer, Hope—and Reason for Worry



OLAGARA, UGANDA—In this harsh, dry landscape, Winifred Omoding's fields are a welcome burst of color. Her neighbors' plots are pitifully brown, with shriveled maize and sorghum clinging to half-height stalks. Omoding's, however, are an embarrassment of green. Her sunflower, sesame, and cassava thrive amid the cacti and dust that surround this village of 500 people.

Just a few years ago, Omoding's prospects looked bleak. Civil war had left her life in disarray, her crops were failing, and she was struggling to feed her family. Now, the 41-year-old farmer not only produces enough food for her husband and nine children but also makes a healthy profit selling the excess.

"I enjoy farming very much," Omoding says as she weeds sunflowers that tower over her head. "It's a very noble profession: the backbone of our country."

It's just the kind of success story that food-security experts say needs to be replicated if fast-growing populations in Uganda and other developing nations are to avoid widespread hunger. Already, analysts estimate that nearly 2 million of Uganda's 31 million people experience food insecurity due to supply problems or rising prices. Nearly 80% of the people in Omoding's region, for instance, depend on food aid to survive. Such problems could worsen as Uganda's population, which has been increasing at

In Uganda, agricultural research is improving food security for some, but not all farmers are prospering

more than 3% per year, surges to an estimated 100 million by 2050. To keep pace, Uganda's farmers will need to at least triple current harvests.

Omoding's story offers some cause for optimism that they can meet that challenge. And it highlights the important role that scientists can play in boosting yields by helping farmers get the most from fundamental resources, such as water (see p. 800), soil (see p. 801), and seeds (see p. 802). But her experience also underscores the complex social, economic, and psychological challenges raised by food insecurity; science alone didn't enable Omoding to transform her fields from brown to green—nor will it do so for her neighbors.

Farming from need

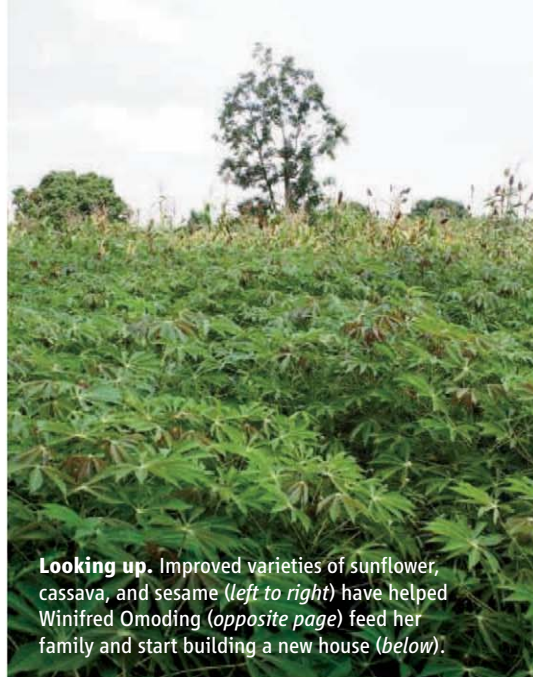
Like many developing-world farmers, Omoding fell into farming out of desperation. Her parents were schoolteachers and she had hoped to follow them into the classroom. But that dream ended with the political violence that enveloped her homeland for nearly 20 years starting in the 1980s. "Whenever we heard shootings, we would run into the bush and hide," she recalls. "The rebels killed my older sister and my dad. They burnt our house, took our seven cows and goats and sheep, destroyed our crops."

After Omoding married, she and her husband, Ephrem, inherited about 3 hectares of land. That is a large farm by Ugandan standards, but the couple struggled through the 1990s. Traditional farming practices, which rarely allow fields to lie fallow, had reduced the fertility of their soil. Poor-quality seeds bought at local markets often failed to thrive. A parasitic weed called striga sapped their sorghum crop, reducing yields. With no animals to help plow the hard ground, the couple "appealed to some of the men in the village, who tied their hands to the harness of the plow," recalls Omoding. "It was a terrible time. Many people went hungry and many children died."

The couple's fortunes changed with the return of political stability in the early 2000s. The men gave up soldiering and could help in the fields. And in 2003, aid groups helped Omoding and other Ologara women form an agricultural "microloan" cooperative. In exchange for making small deposits into the co-op, the women could get small loans. Omoding used her first one to hire oxen to plow and weed her fields.

Such help didn't end the crop failures, however, so in 2006 Omoding traveled to the nearby town of Soroti to seek help from scientists at the government's new National Semi-Arid Resources Research Institute (NaSARRI), created as part of a 2005 overhaul of Uganda's agricultural research system. "She was in a terrible way with her harvest having just failed again," recalls NaSARRI's Florence Olmaikorit-Oumo, an outreach worker who helps connect farmers to institute scientists.

The timing was right. The scientists were developing new crop varieties customized to prosper in places like Ologara, which typically gets less than 800 millimeters of rain annually (and much less



Looking up. Improved varieties of sunflower, cassava, and sesame (left to right) have helped Winifred Omoding (opposite page) feed her family and start building a new house (below).



recently). They were also looking for local farmers to help field-test and multiply the seeds. Omoding was a prime candidate, says Olmaikorit-Oumo: “You could see that she really wanted to learn.”

Institute staff began giving Omoding advice on which crops to grow. Maize was out (too thirsty); sorghum, cassava, and millet were in. They also showed her new ways to restore soil fertility, such as by plowing postharvest leftovers back into the soil. And Omoding got access to the institute’s latest seeds, which she bought using a microloan.

She saw immediate results. The first harvest was so successful that she had a surplus—and a few kilos of desirable new seed—to sell through a marketing network created by NaSARRI. Since then, farm profits have allowed her family to add land, send their children to boarding schools, and start building a brick house. “Before, I farmed to feed my children,” Omoding says. “Now, I think of it as a way to make our lives better and to become more rich.”

Success has also given her the security to experiment with new crops. One is a drought-tolerant sunflower that yields a high-quality oil and a “cake” that farmers can feed to livestock. She’s also planting a new drought-tolerant sesame. “It is ready to harvest in just 4 months rather than 6 months like the local variety,” she says as she wades through a ripe bumper crop.

Duplication challenge

To ensure food security in Uganda, however, many more farmers will soon need to duplicate Omoding’s success. And that could be a problem if the many struggling farms around Olgara are any guide. Even as the Omodings and others have changed their practices and prospered, many neighboring farmers have not—and understanding why will be key to ensuring food security.

Omoding herself believes one important difference is her willingness to take risks and embrace new ideas. “Whatever the scientists tell me, I try it and see if it works,” she says. “I am not happy with just planting the same seeds every year and hoping, like others in the village.”

To overcome that mindset, NaSARRI officials have launched efforts to have innovative farmers teach their neighbors—a model that has worked well elsewhere. But progress has been slow, they say, perhaps in part because so many people here are still recovering from decades of traumatic violence and crop failures that sapped hope for the future. It can seem pointless to put in the hard work necessary to rebuild soil fertility or dig a well, for instance,



if you fear being uprooted from your home or losing your crop to weather or pests you can’t control.

Omoding, however, is looking ahead with confidence. “I always ask how I can do better,” she says. “I want my crops to be bigger.” She wants to plant an orange grove, for instance, to supply a planned juice factory. To get the needed water, she’s already gotten a loan for a treadle pump and is saving up to build a shallow hand-dug well. Eventually, she’d also like to start buying the fertilizers, pesticides, and tractors that farmers in industrialized nations take for granted.

Those dreams, however, rest on a shaky foundation. Part of Omoding’s income, for instance, still comes from aid groups that buy part of her sunflower harvest in order to help jump-start the industry. That income could disappear if the donors withdraw. Reliable water supplies also remain a major challenge, which could get worse with climate change. And experts say Uganda’s government will need to spend much more to develop the infrastructure—from better roads and irrigation systems to reliable banks and markets—needed to give rural farmers incentives to increase yields and connect them to important urban markets.

Still, those trying to ensure food security in Uganda and elsewhere take some hope from Winifred Omoding. If one woman from a small village can create food from the dust, they say, perhaps the challenge of feeding 9 billion of the planet’s future inhabitants becomes a little less daunting.

—GAIA VINCE

Gaia Vince is a freelance writer currently traveling in Africa.

NEWS

Getting More Drops To the Crops

RAJ SAMADHILYA, INDIA—The arid lands around this tiny village in Gujarat don't look promising for profitable farming. But even Raj Samadhilya's poorest farmer routinely reaps generous harvests and owns a sturdy brick house with a flower-filled garden. The picture is quite different in nearby villages, however: Farmers struggle to produce just one harvest a year and rely on government handouts.

The difference is water.

Thanks to a little help from researchers equipped with satellite imagery, Raj Samadhilya's farmers have been able to do a better job of capturing, managing, and using the precious water provided by scanty rains. As a result, they are achieving a goal that scientists say will be essential to achieving food security worldwide: getting more crop per drop, particularly in areas where water could become scarcer due to climate change.

"Successful water management means never being satisfied—every drop is sacred," says Hardevsingh Jadeja, the village chief who catalyzed Raj Samadhilya's water-saving scheme. It's a rare achievement, however, as water management remains a major challenge in dry parts of India and elsewhere. In Africa, for instance, most farmers still depend on unpredictable rains. Just 10% of Africa's farmland is irrigated, compared with 26% in India and 44% in China.

Eye in the sky

Raj Samadhilya shows how melding space-age tools with a few low-tech approaches can dramatically increase water availability.

In 1984, while searching for a better map of his region, Jadeja ended up talking with specialists at the India Space Research Centre in Ahmedabad. They showed him satellite images and maps that revealed the geology underlying his village. The maps highlighted some hidden lineaments—joints or fractures—that run through the rocks. Those cracks, the scientists noted, probably channeled the annual monsoon runoffs to the aquifer beneath. With some careful planning, they added, the town could capture and store some of that water so that it provided a year-round supply to replenish both the aquifer and town wells.

The chief mobilized his villagers. At one promising lineament, they dug down to expose the natural channel. Then they dug a 20 meter by 30 meter reservoir to capture the seasonal flow—high enough so that gravity would slowly channel the water down to the aquifer rather than running off. Perhaps most importantly, Jadeja used his political skills to pass some new community rules. Farmers adopted irrigation techniques that don't waste water, such as pipes that deliver tiny drips directly to



Wet wealth. Stored water has helped lift villagers out of poverty and into commerce.

plant roots. And nobody is allowed to draw directly from the precious reservoir, which is also used for watering cattle.

It worked. Long after the annual monsoons ended, the stored water helped maintain groundwater levels during dry

spells. Wind-, hand-, and oxen-driven pumps distributed the water through the drip tubes to about 100 hectares of fields. Ultimately, even the village's more than 100 households got piped water and toilets. And the government water tankers that once made routine deliveries during the dry season no longer stopped at the village.

Now, Jadeja is moving to make the system even more efficient. Recently, as he showed off a drip-fed bean field planted with its third crop of the year, he discussed plans to reduce the reservoir's surface area by making it deeper, in order to cut losses from evaporation. "Saving those drops means the difference between a hungry child and one that is educated and ready to help improve the country," he says.

Drop by drop

In other poorer or dryer parts of the world, farmers are also learning to make the most of meager rains. In arid eastern Uganda, for instance, government researchers are teaching ground nut (peanut) farmers to place their plants in raised ridges or earthen mounds so that the rain-water soaks into the roots rather than running off. Other approaches include piling rocks around crop plants to help hold in moisture and teaching farmers that they can withhold water from some crops during certain growth stages without harming yields.

Some aid organizations, meanwhile, are drawing on their experience helping villages build simple drinking water systems to bring more water to farmers. In Kihonda, Uganda, for instance, last October the Busoga Trust, a U.K. nonprofit, helped villagers install a hand-powered pump. Some villagers are already watering their gardens, although it's an arduous process because they have to carry the water in plastic "jerrycans," the ubiquitous 20-liter yellow canisters seen across Africa. In communities that don't have access to well-drilling machinery, villagers are hand-digging wells if the water table is high enough.

Such approaches may be less effective than direct irrigation. But they reflect the reality that although larger-scale water diversion schemes have worked well in places like India, they are still out of reach for many African communities. "We can tell them to use channel irrigation," says Patrick Rubaihayo, a crop scientist at Makerere University in Kampala, Uganda. "But irrigation systems are very expensive to maintain by an ordinary farmer." Ultimately, he says farmers will need government help to expand irrigation, particularly in sub-Saharan Africa. There, just 4% of land is irrigated, and scholars say irrigation has actually decreased over the past 30 years because projects built during the colonial era have fallen into disrepair.

In Uganda, the government hopes to reverse that trend. It recently announced plans to rebuild dozens of crumbling "valley dams" that were built in several dry regions in the 1970s to trap rainwater for farmers raising cattle.

—GAIA VINCE

Gaia Vince is a freelance writer currently traveling in Africa.

NEWS

China's Push to Add by Subtracting Fertilizer

CUIDONGGOU, CHINA—In his first stab at growing tomatoes, Meng Heini hit the jackpot. Two months after transplanting an inaugural batch of seedlings, his greenhouse is packed with vines laden with small green globes. The tomatoes are thriving, Meng says, because he generously coats his soil with a mixture of manure and synthetic nitrogen fertilizer. “If you use a lot, you get high yield,” he says.

That idea has become nearly gospel among China's 350 million farmers. Since 1977, they have nearly tripled fertilizer use, in part urged on by government subsidies and local officials who take kickbacks from fertilizer sales. Today, China is the world's largest user, consuming 36% of the global total of synthetic fertilizer.

The increase has helped farmers nearly double grain harvests. But it also exceeded soil needs, causing nitrates to accumulate and create serious pollution problems. And the hunger for nitrogen has added to China's energy and greenhouse gas emissions: In the atmosphere, those nitrates form nitrous oxide, a potent warming gas.

Now, as the country attempts to coax even bigger harvests from the land, soil scientists want to end China's fertilizer binge. Through several promising demonstration projects, they are showing farmers that reducing fertilizer use can improve crop yields without adding to environmental problems. The new maxim, say Chinese soil scientists, is “Less input, more output.”

Surprisingly, that strategy—making less fertilizer go further—could also hold promise for farmers in nations with the opposite problem: too little fertilizer but a big need to increase soil fertility. The common solution? Helping all farmers get “high yield and high efficiency” in fertilizer use, says Zhang Fusuo, a soil scientist at China Agricultural University (CAU) in Beijing.

Early resistance

That message initially proved a hard sell in Cuidonggou, a village near Meng's greenhouse. When Chinese and British scientists funded by the United Kingdom's Department for International Development arrived in 2007 looking to recruit farmers willing to cut fertilizer use on half of their land, “at first no one believed what they said,” recalls 61-year-old farmer Cui Tao. The scientists were calling for reducing fertilizer use by an average of 30% on winter wheat fields and 50% on maize fields. But farmers signed on after the scientists promised compensation, providing a cushion if yields fell.

The next harvest proved researchers right. Yields from the 30 experimental plots were identical to or better than yields from higher-input fields. Farmers who adopted the low-nitrogen approach boosted profits by 1200 yuan (\$176) per hectare, a sizable sum. Projects elsewhere produced similar results. Farmers in the Taihu and North China Plain regions have cut fertilizer use by 30% to 60% without reducing yields.

Now, researchers want to encourage farmers to try other low-fertilizer tricks. For instance, by splitting applications into two smaller batches—one before planting and one when plants are growing fastest—farmers can deliver nutrients when the crops need them most.

Shifts in China's rural economy may hinder the spread of such ideas, however. Many young farmers now head off to construction or factory jobs in the city after sowing their fields. That means they don't have time to apply nitrogen in multiple doses, and “increasing fertilizer efficiency takes time,” says Huang Jikun, director of the Center for Chinese Agricultural Policy at the CAU.



Cutting back. Soil scientists such as Tong Yanan (*right*) have persuaded many farmers to use less fertilizer, but some continue to pile on manure (*inset*).

Other farmers are simply unwilling to risk cutting back. Tomato farmer Meng, for instance, pays a hefty rent for his greenhouse. So, “as much fertilizer as I have, that's how much I use,” he says proudly.

Scientists also caution that the lessons learned here have limits. One reason some farmers aren't seeing lower yields, for instance, is because years of pollution and indiscriminate fertilizer use have left China's air, water, and soils overstocked with nitrogen. “At the moment, you can use up that stock in the camel's hump,” says David Powlson, a soil scientist at Rothamsted Research, an agricultural institute in Harpenden, U.K., and lead scientist in the Cuidonggou project.

Still, researchers believe China can trim its fertilizer habit. Overuse has been a rite of passage for many industrialized countries, they note, including the United Kingdom, where use peaked in the mid-1980s; yields have since gone up despite cuts. China now has a chance to follow the same path, says David Norse, a professor emeritus in environmental management at University College London. “We want to help them get away from this fear that by reducing fertilizer they're going to be damaging food security,” he says.

Abundance in scarcity?

For other parts of the world, China's problems are a luxury. In sub-Saharan Africa, which uses a tiny fraction of the nitrogen China applies, chemical fertilizer is expensive and livestock manure scarce. But there, too, researchers are banking on the idea that less can mean more through an approach called “microdosing.”

In recent trials in Zimbabwe, for instance, researchers from the International Crops Research Institute for the Semi-Arid Tropics in Malawi have shown that crops can thrive with as little as a thimble-full of fertilizer applied close to the roots at the time of sowing. Those microdoses boosted yields of maize, sorghum, and pearl millet by 30% to 100%.

Scientists say African farmers may also be able to minimize fertilizer use by improving irrigation and using better seeds, and—eventually—through new approaches to promoting the growth of beneficial soil bacteria. The world's farmers “need fertilizer, but not only fertilizer,” says CAUs Zhang. “What they really need is integrated management.”

That's a lesson China and other countries learned the hard way, Zhang says, adding that farmers elsewhere “do not need to repeat our mistakes.”

—MARA HVISTENDAHL

Mara Hvistendahl is a writer in Shanghai, China.

NEWS

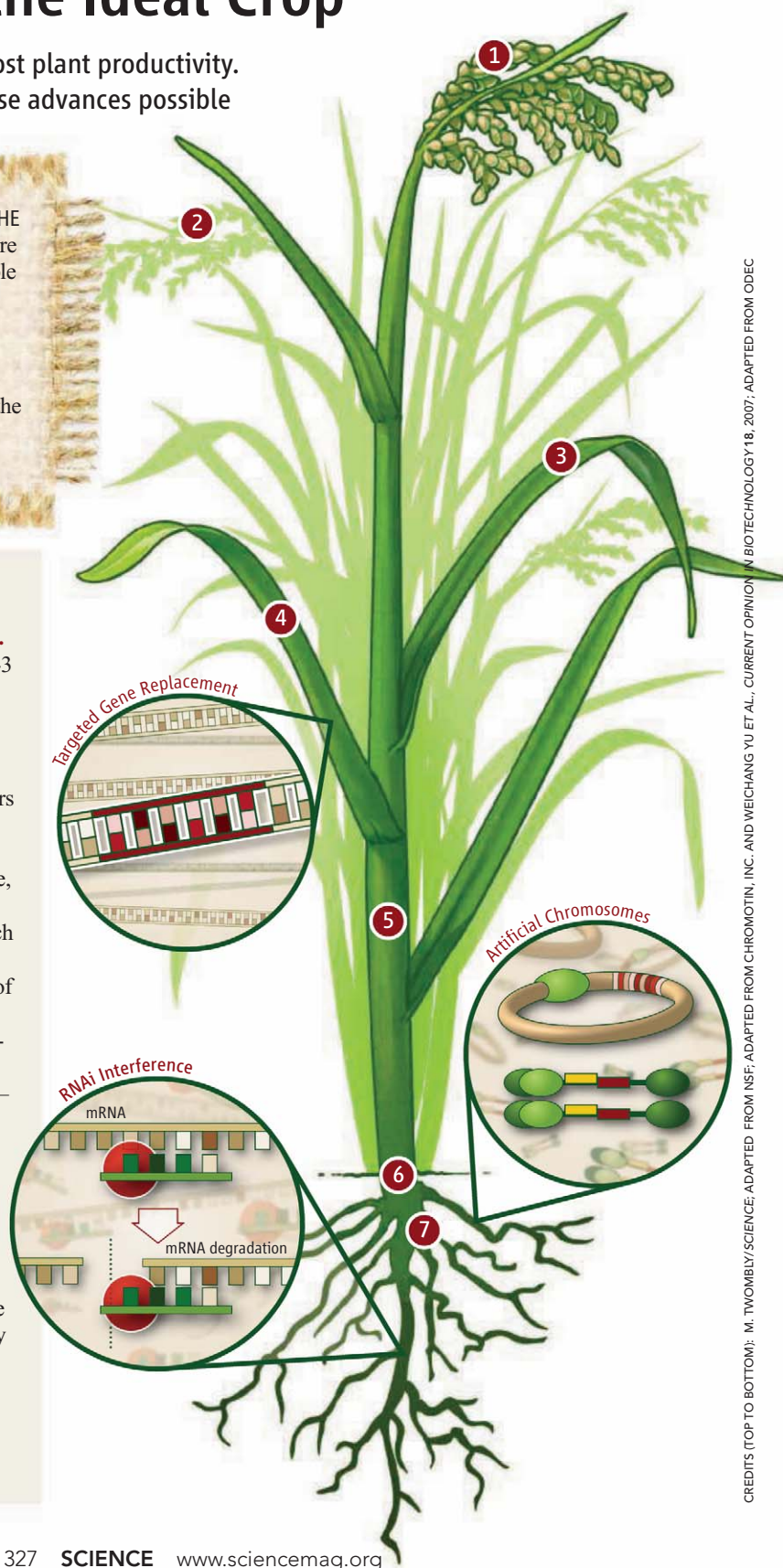
Sowing the Seeds for the Ideal Crop

Researchers' wish list includes traits that could boost plant productivity. New technologies are needed to make some of these advances possible

LISTEN TO PLANT BREEDERS TALK ABOUT FOOD SECURITY, AND THE message becomes loud and clear: Substantial improvements are needed in current crops to achieve higher yields and sustainable farming. To achieve those gains, agricultural companies have turned to robotics and other measures to streamline breeding programs. And researchers are finding creative ways to introduce and use genes. The point is to make a plant that's tough, productive, and healthful. Here's a quick look at just some of the most desired plant improvements—and the techniques that might make them possible.

LOFTY GOALS

- 1 Improve the nutrient content of seeds and edible plant parts.** Vitamin A fortification is already here; soybeans with omega-3 fatty acids are on the way. More vitamins and higher protein content are other goals. For biofuels, the right mix of plant cell-wall components is needed to ease processing.
- 2 No more sex.** Hybrid seeds often produce more vigorous plants, but the seeds of those hybrids are often inferior. Farmers can't always afford to buy new hybrid seeds. One proposed solution is to get hybrids to reproduce asexually, through a process called apomixis. Having apomixis in rice, for example, could save small farmers \$4 billion a year. (An alternative to apomixis is to tweak the genetics of annual crop plants—which die each year—so that they become perennials.)
- 3 Install warning lights.** A pigment gene that turns on in times of stress could cause a crop's leaves or stems to change color—and alert farmers to take remedial action. Some think that sensors installed in soils or the air could also do this job.
- 4 More crop per drop.** Restructuring root and leaf architecture—and upgrading drought-response biochemical pathways—could increase water-use efficiency. Shallower roots, for instance, can better tap soil-surface moisture.
- 5 Longer shelf life.** Enhanced control of ripening and senescence could reduce the amount of spoiled harvest.
- 6 Improve nitrogen efficiency.** Fertilizers are costly to farmers and the environment. Improving a plant's uptake and use would be a big help. Better yet, build into the plant the genes necessary to carry out nitrogen fixation—a job that may one day fall on artificial chromosomes.
- 7 Tougher pest defenses.** Adding genes for toxins that kill only pest insects or nematodes can help, as can the addition of genes that attract the enemies of these pests.



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TECHNOLOGIES FOR A BETTER FARMING FUTURE

Artificial Chromosomes

If one gene is good, more genes are better. That's the mantra of plant biologists working to improve crops. Already, companies have engineered varieties that carry both herbicide and insect-resistance genes. Ultimately, researchers have set their sights on tweaking complex multigene processes, such as nitrogen fixation, which might involve 20 genes, or a special type of photosynthesis called C4 that works particularly well in tough conditions. Coordinating the expression of whole suites of genes, however, is an easier feat if the genes are grouped together. Here's where artificial chromosomes come into play.

Such "minichromosomes" come in several flavors. A company called Chromatin, for instance, has developed a way to attach useful suites of genes to a "platform" made from a ring of maize DNA. It encodes the repetitive regions of the centromere, the region near the middle of chromosomes that is important during DNA replication. Once loaded with the desired genes, the ring is put into the target plant.

Several teams are also making use of a plant's own "extra" DNA—such as the B chromosome in maize, or extra chromosomes in tetraploid versions of barley, rice, or *Arabidopsis*.

They insert DNA containing the desired genes and the repetitive

sequence of a telomere, which caps off chromosomes. That DNA inserts into the plant's chromosome and truncates it, creating a new minichromosome.

These techniques are promising, but it's not clear how stable the minichromosomes will be over multiple generations—or if the right amount of gene expression will be maintained over time.

RNA Interference

One innovative approach to helping plants fend off enemies is to have the plant make small RNA molecules that neutralize the pathogen or pest. Called RNA interference (RNAi), the strategy involves adding genes that generate RNA that disables target genes. These small RNAs can diffuse from the plant into a parasite or deactivate viruses in the plant itself.

Researchers have already shown that they can use RNAi to make *Arabidopsis* plants resistant to the root-knot nematode and reduce the symptoms of cassava mosaic virus by interfering with genes for a protein essential for virus replication. RNAi also should work against other so-called single-stranded DNA viruses, such as the cassava brown streak. Other researchers have used RNAi to shut down a detoxification gene in bollworm, a cotton pest—making the insect more susceptible to gossypol, a toxin produced by cotton.

Still, a National Research Council report from 2008 called RNAi's potential "preliminary," particularly with respect to large-scale agriculture.

Targeted Gene Replacement

Researchers typically fly blind when they try to add or modify plant genes, because they can't be sure where the gene they are inserting will end up. They often have to laboriously screen thousands of mutants to find the right one. Now, however, researchers are perfecting ways to place new genes precisely where they want them. These techniques could ease the process of improving crops by allowing researchers to alter a particular gene's sequence or its regulatory DNA instead of depending on genes from other species.

In one new approach, several groups are harnessing proteins called zinc fingers, which recognize and attach to specific DNA sequences. By joining the zinc finger to an enzyme that cuts DNA, researchers can slice through DNA at precise locations, providing ready places for new DNA to settle in.

Other groups are taking a cue from a bacterial pathogen, *Xanthomonas*. Late last year, two teams discovered how this pathogen promotes infection by homing in on and controlling specific plant genes. The bacteria make a protein with an amino acid sequence that includes a series of "repeats." Each repeat has a particular amino acid at positions 12 and 13 and thus recognizes a specific base, enabling the protein to latch onto its target DNA. Both teams have figured out how to customize these proteins to find specific DNA sequences, potentially providing another way to add new genes or make modifications to specific parts of a chromosome.

A third approach involves endonucleases, which naturally cleave DNA at specific spots. These proteins recognize long stretches of DNA and can target unique spots along a genome.

Robotics

Automation is helping plant breeders trim years off the process of developing crop varieties tailored to local conditions. In automated greenhouses, seedlings travel along conveyor belts as they grow, passing through stations where they are exposed to drought, heat, or other conditions. The plants are then photographed and monitored, all without human help. The controlled conditions help breeders find individuals with the best traits without field testing.

Automated systems are also helping breeders skip the time-consuming process of growing seedlings as they search for desirable genetic combinations. Monsanto, for example, has developed "chippers," robots that take a slice off individual corn kernels or soybeans—sparing the germ that's the business end of the seed. The slice goes to the lab for DNA fingerprinting to determine its mix of genes; the rest of the seed is carefully stored. Later, if breeders are interested in that genetic variant, they can electronically request and automatically retrieve the stored seed. They no longer have to wait for seeds to sprout to see which ones have the traits they want.

—ELIZABETH PENNISI



Add-on. The isolated green dot marks the centromere of a minichromosome.



Time-saver. This "corn chipper" samples DNA from seeds.

NEWS

Armed and Dangerous

These fungi, weeds, and viruses are among the more serious biological threats to food security—so researchers are working hard on countermeasures



WHEAT STEM RUST

Pest: *Puccinia graminis* Ug99

Crop: Wheat

Whereabouts: Fifty years ago, stem rust led to the resistant wheat varieties that fueled the Green Revolution—leading many farmers to believe they were done with *Puccinia graminis*. But in 1998, a dangerous new strain named Ug99 appeared in Uganda (*Science*, 30 March 2007, p. 1786). By 2004, its spread prompted Green Revolution pioneer Norman Borlaug to launch a global research initiative to address the threat. Ug99 has since shown up in Yemen and Iran and threatens wheat crops throughout the Middle East and West Asia. The big fear: Ug99 could cause famine in Pakistan and India, where small farmers can't afford the fungicides used to control the disease.

Symptoms: The fungus infiltrates stems and plugs up vascular tissue. Of the three common rust diseases, stem rust is the worst because it causes the plant to fall over, so the entire harvest is lost.

Losses: Heavy infections can reduce yields by 40% or more. If it reaches India's Punjab region, losses could reach \$3 billion per year; if it reaches the United States, the toll could be \$10 billion annually.

Countermeasures: The International Maize and Wheat Improvement Center in Mexico has created 15 resistant wheat varieties, but Ug99 is infamous for quickly overcoming resistance.



POTATO BLIGHT

Pest: *Phytophthora infestans*

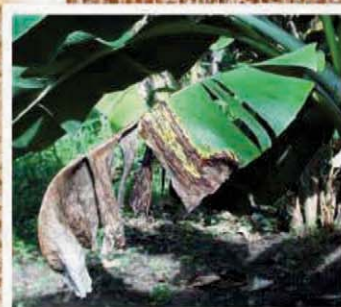
Crops: Potatoes; also tomatoes and other solanaceous crops

Whereabouts: This funguslike organism occurs wherever farmers grow potatoes.

Symptoms: Most notorious for causing the Irish potato-famine of 1845 to 1851, late blight still ranks as the world's most dangerous potato disease. Spread by spores or by planting infected tubers, it first appears as gray splotches on leaves. In high humidity and moderate temperatures, it can destroy a whole field in a week.

Losses: The International Potato Center in Peru reports that yield losses in developing countries are about \$2.75 billion annually. Fungicide applications can total 10% of overall production costs.

Countermeasures: Fungicides work but can be harmful to human health and too costly for poor farmers.



BLACK SIGATOKA

Pest: *Mycosphaerella fijiensis*

Crops: Bananas, plantains

Whereabouts: This fungus, first detected in Fiji in 1964, is now found in 100 countries in the Americas, Africa and South Asia.

Symptoms: The fungus starts as small flecks on the undersides of the youngest leaves. They expand into brown streaks that can eventually destroy the leaf, decreasing photosynthesis. Fruit from diseased trees can ripen prematurely during shipping, causing further losses.

Losses: Yields reduced up to 50%.

Countermeasures: Commercial plantations frequently apply cocktails of fungicides, sometimes from airplanes, and remove leaves at a cost of 15% to 50% of the fruit's final retail price.



WITCHWEED

Pest: *Striga hermonthica*

Crops: Corn, sorghum, sugarcane, millet, native grasses

Whereabouts: *Striga* originated in Africa and has since become widespread in the tropics.

Symptoms: This parasitic plant attaches to the host's roots, where it siphons off nutrients and water, stunting the host's growth and causing it to wither. When *Striga* emerges aboveground, it makes a substance toxic to the host. One plant can produce 50,000 tiny seeds that stick to people and their tools or settle in the soil. Seeds can stay dormant for 15 years.

Losses: In sub-Saharan Africa, *Striga* infects 20 million to 40 million hectares, reducing yields by 20% to 100%. Losses total about \$1 billion per year and affect 100 million people.

Countermeasures: Some *Striga*-tolerant maize can produce small ears despite being parasitized. But farmers must scramble to destroy plants before they produce seed and plant nonhost crops in affected soils. Another approach is to plant a legume called *Desmodium*, which secretes a chemical that kills *Striga*, but that requires using livestock to control the *Desmodium*. Researchers are looking into applying a fungus to kill the seeds.



RICE BLAST

Pest: *Magnaporthe oryzae*

Crops: Rice, 50 species of grasses and sedges

Whereabouts: Worldwide

Symptoms: Spores infect plants, particularly when humidity is high, often killing young plants. In older plants, the fungus can spread and prevent seed formation.

Losses: Destruction can be extremely fast but variable, with up to 100% loss in some paddies. Some analysts estimate that each year blast destroys harvests that could feed 60 million people, at a cost of some \$66 billion.

Countermeasures: Rice blast is a formidable foe, persisting despite the best control efforts. Farmers can manage the disease by rotating crops, maintaining water levels (too little water promotes infection), and using fertilizers prudently. Resistant cultivars help, but no cultivar can withstand all races of the fungus, and blast tends to overcome resistance in two or three growing seasons. Farmers can also use fungicides.



SPECIAL SECTION

ASIAN SOYBEAN RUST

Pest: *Phakopsora pachyrhizi*

Crops: At least 31 legume species, notably soybeans

Whereabouts: Native to Asia, soybean rust spread to Australia in

the 1980s and reached Africa a decade later. It hit South America in 2001, and Hurricane Ivan carried spores into the United States in 2004. It's now found throughout the Southeastern United States and Mexico (*Science*, 3 December 2004, p. 1672).

Symptoms: Infected plants develop small pustules on the undersides of leaves that spread throughout the plant. In the United States, the invasive vine kudzu is the primary host and vector for soybean rust.

Losses: Yields reduced 10% to 80%.

Countermeasures: Early detection and multiple applications of fungicide.



CASSAVA BROWN STREAK VIRUS

Pest: Virus

Crops: Cassava, also called yucca, manioc, and mandioca

Whereabouts: East and Central Africa

Symptoms: This virus is emerging as a major threat to a crop already under siege from cassava mosaic virus. Spread by whiteflies and by cuttings, brown streak virus is more insidious than the mosaic virus because the plant can look healthy even as the disease destroys the edible root. Once confined to lowlands in East Africa, it appeared in Uganda in 2004 and has become a threat throughout sub-Saharan Africa. Disease often appears where farmers have planted cassava varieties resistant to mosaic virus.

Losses: Yields drop by up to 100%. In 2003, economic losses totaled more than \$100 million per year. This virus and cassava mosaic virus have been called Africa's biggest threat to food security.

Countermeasures: The International Institute of Tropical Agriculture, based in Nigeria, is developing tolerant varieties whose leaves become diseased but whose roots stay healthy. Early-warning monitoring programs and early harvesting can help reduce the impact of the diseases.

—ELIZABETH PENNISI



Sign of destruction. Brown swaths indicate the extent of rodent damage in rice terraces in the Philippines.

NEWS

Holding Back a Torrent of Rats

A “RAT FLOOD.” THAT’S WHAT THE TRIBES IN BANGLADESH’S CHITTAGONG Hill Tracts call it. Every 48 years, the bamboo forests that dominate the uplands of Bangladesh, Northeast India, and Myanmar (formerly known as Burma) simultaneously produce a feast of pear-sized fruit that allows rat populations to explode. After consuming the fruit, the rodents attack nearby fields, devouring 50% to 100% of the rice crop. Rat floods caused famine in 1863, 1911, and 1959, when the misery touched off a rebellion in what is now India’s Mizoram State.

Rat floods may be unusual, but rodent losses are a perennial problem worldwide. In Asia, for instance, rodents devour an estimated 6% of the annual rice harvest—roughly enough to feed Indonesia’s 240 million people for a year. And they do damage in nearly every phase of farming, from munching on seedlings to eating stored grain.

Many farmers and agricultural officials, however, shrug. “Philippines farmers say, ‘For every 10 rows of rice we plant, seven are for the family, two for the rats, and one for the birds,’ ” says Grant Singleton, a wildlife ecologist at the International Rice Research Institute in Los Baños, Philippines. Rat fatalism runs so deep that agricultural universities, which have courses in insect management, offer no training in defending against rodents. Thanks in part to growing concerns about food security, however, Singleton says rats are now “getting on the radar.”

Rat race

In the wake of that recognition, agriculture agencies across Asia have started spreading the word about some relatively simple rat countermeasures. Small-scale farmers, for instance, often store

grain in open bins in their homes and “don’t appreciate what [rats] are taking,” says Singleton. Steps such as raising the bin off the floor and installing metal flashing around bin legs can cut losses.

Rat fighters are also urging all farmers within a community to plant their crops within 2 weeks of each other. If fields ripen together, grain is available for a shorter time and rodents curtail breeding. Communities can also maximize efforts to flush out, trap, and kill rats by launching campaigns before planting begins. When paddies and fields are fallow, rodents tend to congregate in the thickets between fields and along roads and irrigation channels. “While they are aggregated, they are much easier to control,” says Singleton. Most important, he says, communities need to work together: “If you do everything we think should be done to manage rodents and your neighbor does not, you will inherit those rodents.”

Some of the 200-plus species of rats that pester farmers, however, require carefully timed control strategies that reflect unique habits. For instance, Indonesia’s rice field rat, *Rattus argentiventer*, does its worst damage just as grains start to form, because the rats must eat huge quantities of immature grain to get sufficient nutrition; as the grain ripens, they eat less. In contrast, Myanmar’s *Bandicota bengalensis* rats cause little damage until just before harvest, when they grab all the grain they can to hoard in burrows. “The dynamics of the damage differs by species,” Singleton says.

Rodents also respond to unusual patterns of food availability. In May 2008, Cyclone Nargis devastated the rice crop in Myanmar’s Ayeyarwady delta (*Science*, 8 May 2009, p. 715). To recover, farmers planted rice when and where they could. As a result, the rice ripened at different times in neighboring paddies—providing a steady food supply for rats. The rodents bred for longer than usual, leading to a surprise outbreak this year that further dented precarious food supplies.

All together now

Even recognized events such as bamboo fruiting, however, can be difficult to prepare for. One problem is that agricultural agencies are reluctant to fund the long-term studies needed to understand the connection between bamboo “masting,” where an entire population produces fruit simultaneously, and rodent explosions. Masting can occur at intervals ranging from several years to more than 100 years, depending on the species, so “there are few opportunities to study this,” says Steven Belmain, an ecologist at the Natural Resources Institute of the University of Greenwich in Chatham Maritime, U.K. Only over the last several years, for instance, have scientists unraveled what happens to rat populations when masting occurs in *Melocanna baccifera*, which makes up more than 80% of the bamboo in Bangladesh, India’s Mizoram State, and Myanmar.

Typically, rodents in that region start breeding in April or May, after the dry season when the first monsoon rains allow food in the form of insects and plants to proliferate. Upland farmers plant their rain-fed crops at the same time. Rodent populations build through the summer and damage the harvest, but losses are usually manageable. Once every 48 years, however, the *Melocanna* bamboo starts dropping fruit in February. With food abundant, the rodents start breeding 2 to 3 months earlier than usual. This head start means that “multiple generations of rats are breeding, [producing] exponential growth in the population,” says Ken Aplin, a wildlife biologist at Australia’s Commonwealth Scientific and Industrial Research Organisation in Canberra. By autumn, just as crops are ripening, the food in the bamboo forest is gone, leading to “a mass movement [of rodents] from the

forest into the fields,” says Aplin, who advised the Mizoram state government on dealing with masting. But “there is no way to stop the ecological phenomenon,” says Belmain. “You can only manage the damage.”

Anticipating the 2008 *Melocanna* masting event, for instance, the Mizoram government launched the 5-year Bamboo Flowering and Famine Combat Scheme that included upgrading roads to carry aid to remote communities, rat-proofing warehouses, and encouraging farmers to plant early-yield rice varieties and alternative crops less attractive to rodents. When the inevitable rat flood hit, the government and relief organizations provided food assistance. “In a broad sense, it worked,” says Aplin, though it will take several years for the area to completely recover.

Now, researchers are pondering how the lessons learned could help other regions. If researchers can pin down when and where masting events will occur, “it might allow us to understand which communities will be hit so limited resources can be better targeted,” says Belmain. Rat flood control, it seems, is just getting started.

—DENNIS NORMILE



Cute but hungry. Researchers track rats fitted with transmitting collars to understand their movements and habits.



NEWS

Spoiling for a Fight With Mold

IT'S TOUGH GETTING PEOPLE TO WORRY ABOUT MOLD AND ITS ROLE IN food security. “Everyone has seen mold on things in refrigerators and says, ‘It’s just mold, it doesn’t matter,’” says John Pitt, a fungus specialist at Australia’s Commonwealth Scientific and Industrial Research Organisation (CSIRO) in Sydney. But mold spoils some 10% of the world’s annual harvests, he notes. And perhaps more significantly, fungal toxins in food “are certainly having a major impact on life spans in developing countries. It’s an area which doesn’t get anything like the publicity it should.”

That is certainly not for lack of Pitt’s efforts. He has focused virtually his entire 45-year career on understanding fungi and trying to reduce the losses they cause—and gained renown in the process.

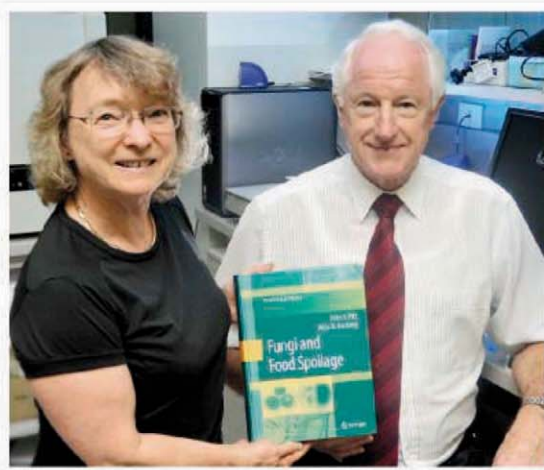
Pitt graduated from high school at 16 in 1953 and immediately got a job with CSIRO’s Division of Food Preservation and Transport. He got hooked on fungi at a time when what he calls the “fascinating” organisms, which can leave crops putrid and inedible, got little attention from agricultural experts. Eventually, his work determining which fungi infected which crops, their origins, and developing techniques to measure infection levels practically established a new field. In 1985, along with longtime CSIRO colleague Ailsa Hocking, he distilled his findings into a thick tome—*Fungi and Food Spoilage*—“that was a

milestone” in food safety, says Antonio Logrieco, a mycotoxicologist at the Institute of Sciences of Food Production in Bari, Italy. The third edition appeared last August.

Pitt has also helped raise the alarm about insidious health effects. Many fungi produce mycotoxins, poisonous chemicals that can accumulate in human tissues. The most dangerous is aflatoxin, which Pitt calls “by far the worst liver carcinogen known to man.” Two fungus species produce aflatoxin in peanuts, maize, and cotton seeds if the crop is stressed by drought or stored improperly. In advanced countries, inspection and testing weeds out infected material. But subsistence farmers in developing nations often aren’t aware of the threat. The result, Pitt says, is that the toxin is elevating rates of liver cancer and likely stunting childhood growth in Africa, Southeast Asia, and China. Aflatoxin “probably has a much bigger effect on human health than has ever been fully documented,” Pitt says. He’s now working with the World Health Organization’s Foodborne Disease Burden Epidemiology Reference Group to quantify the toll.

Pitt is also studying a possible solution. It’s based on the concept of “competitive exclusion,” which involves introducing spores of a benign fungus into the soil in hopes it will out-compete and drive out the aflatoxin-producing strain. But these days, he’s doing it on his own. As an Honorary Research Fellow at CSIRO he has lab space but no longer draws a salary; he even pays his own way to international meetings. It’s expensive, but Pitt says he’s still just trying to get fungi the attention they deserve.

—DENNIS NORMILE



Fungi fighters’ bible. Ailsa Hocking (left) and John Pitt co-wrote the standard reference book on fungi that spoil and contaminate food.

CREDITS: (TOP) PETER BROWN; (BOTTOM) CHRIS TAYLOR

NEWS

Dialing Up Knowledge—and Harvests

EMBALAM, INDIA—In the open-air hall of a Hindu temple in this village in southern India, five farmers in smart white cotton shirts sit cross-legged on a carpet and explain how cell phones have changed their lives. A few years ago, says Poonathan, who like many in Tamil Nadu State has only one name, “I would have to drive into town to check the price that rice was fetching or find out where to buy high-quality seed.” These days, like many other farmers across rural India, he instead stays home and dials a cell phone to get everything from the weather forecast to primers on how to use less seed, fuel, and fertilizer but still reap bigger harvests.

“We now have money to spend on our children’s education, and many of us don’t need to borrow anymore to buy seed and fertilizer,” says a second farmer, Krishnaswamy, who with dusk falling swats at mosquitoes attacking his bare calves. Few of the 5000 or so inhabitants of Embalam miss out on three meals a day, he adds—an impressive accomplishment in light of India’s 200 million or so malnourished people. Krishnaswamy nods to a group of worshippers lighting candles in front of a statue of the monkey god Hanuman, praying for good fortune. “We consider ourselves lucky,” he says.

From paved roads that carry crops to market to modern grain silos that reduce postharvest losses, infrastructure is critical to achieving food security. But nothing is currently having a more profound effect on farmers in the developing world than telecommunications networks. “The future of food security in the developing world depends more on knowledge than on resource-intensive agriculture,” says Venkataraman Balaji of the International Crops Research Institute for the Semi-Arid Tropics in Patancheru, India. In Tamil Nadu, for instance, the ubiquitous cell phone and expanding broadband Internet coverage are revving up an experiment called Village Knowledge Centers (VKCs), the template for an ambitious initiative to help farmers boost yields by disseminating information. A similar effort has just gotten under way in India’s northern neighbor, Bhutan.

“Cell phones are responsible for an amazing transformation,” says Anburaj Thiagarajan, a Puducherry-based adviser to the Jamsetji Tata

National Virtual Academy for Rural Prosperity, a forum for honoring social workers, farmers, and others who have made a difference to their home villages. The transformation is going on speed dial: India’s government intends to launch up to 100,000 new VKCs by the end of 2012. Pulling off that feat won’t be easy, says Uma Lele, a former senior adviser to the World Bank. “It is a daunting challenge to get communities actively involved when scaling up a carefully nurtured pilot project,” she says. Meanwhile, Green Revolution pioneer M. S. Swaminathan is prodding the government and private companies to recruit tech-savvy volunteers in each of India’s 600,000 villages who, he says, can “get science into the hands of more people.”

Getting the word out

The M. S. Swaminathan Research Foundation (MSSRF) in Chennai opened the first VKCs in Tamil Nadu in 1998 to spread the fruits of agricultural research to farmers. The centers started off as spartan offices that distributed pamphlets and offered hands-on training. At the time, “nobody could see the applicability of mobile phones to rural life,” says Suchit Nanda, a Mumbai-based consultant. The couple of dozen VKCs here have since morphed into multimedia centers that communicate with each other via broadband and send dispatches on info such as commodity prices to farmers via cell phone.

It’s a model that’s being emulated by Bhutan’s new Market Information System. Last month, the Netherlands Development Organisation (SNV), Bhutan’s agriculture ministry, and Bhutan Telecom established the network, which allows farmers to use cell phones to get daily price ranges of major crops in five market centers.

The system aims to ameliorate a common problem in isolated farming communities, which in mountainous Bhutan means almost everyone: the temptation of middlemen to rip off farmers who are not aware of prevailing prices. “A lot of farmers are being caught like that,” says Rob Erskine-Smith, an SNV consultant based in Thimphu who helped develop the system. By dialing up timely prices, farmers will be able to negotiate better deals with middlemen and commission agents—and capture more of the cash needed to improve their operations. Unlike similar setups that use text messaging, the Bhutan system recites prices in the country’s four main languages—a big advantage in a country with a high rate of illiteracy. The key to long-term success will be ensuring that prices collated by the agriculture ministry and Food Corporation of Bhutan are not manipulated to favor buyers or sellers.

To provide wiser counsel to Indian farmers, Swaminathan is raising an army of village volunteers. In 2005, with support from the Tata family, prominent Indian industrialists, MSSRF created the Jamsetji virtual academy, whose 1000-and-counting members receive MSSRF-led training and share experiences at annual gatherings. “MSSRF has been able to identify ordinary people and enable them to do extraordinary things,” says Nanda.

Swaminathan dreams that the academy will eventually tap at least one man and one woman from each village—some 1.2 million people. As villages acquire VKCs and get better connected thanks to cell phones and the Internet, information should flow easily from researchers to farmers and from farmer to farmer. It’s the kind of infrastructure that Swaminathan hopes will help India build on the Green Revolution in the face of a growing population—and climate change. —RICHARD STONE



Telecom revolution. In Bhutan, a new cell phone service should help farmers fetch better prices for produce. Cell phones have already transformed life for farmers in Embalam, India (*inset*).

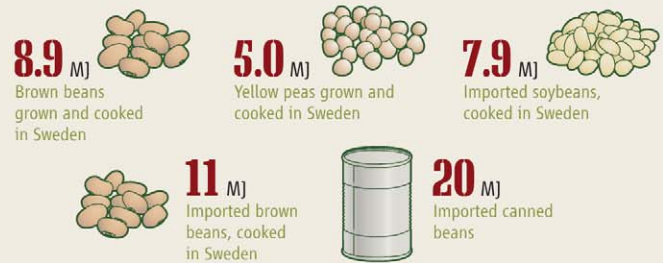
NEWS

What It Takes to Make That Meal

FOOD SECURITY AND ENERGY SECURITY. THEY ARE INCREASINGLY becoming two sides of the same coin. Many experts predict that, over the long term, one can't be achieved without the other. In part, that's because increasing yields has traditionally meant using more fossil fuels—for fertilizers, pesticides, mechanization, storage, and transport. Now, the push is on to find ways to produce food with as little energy—and greenhouse gas emissions—as possible. As a start, researchers have been taking a close look at just how much energy it takes to produce even seemingly similar foods. The conclusion: Food choices can have a significant impact on energy use in agriculture.

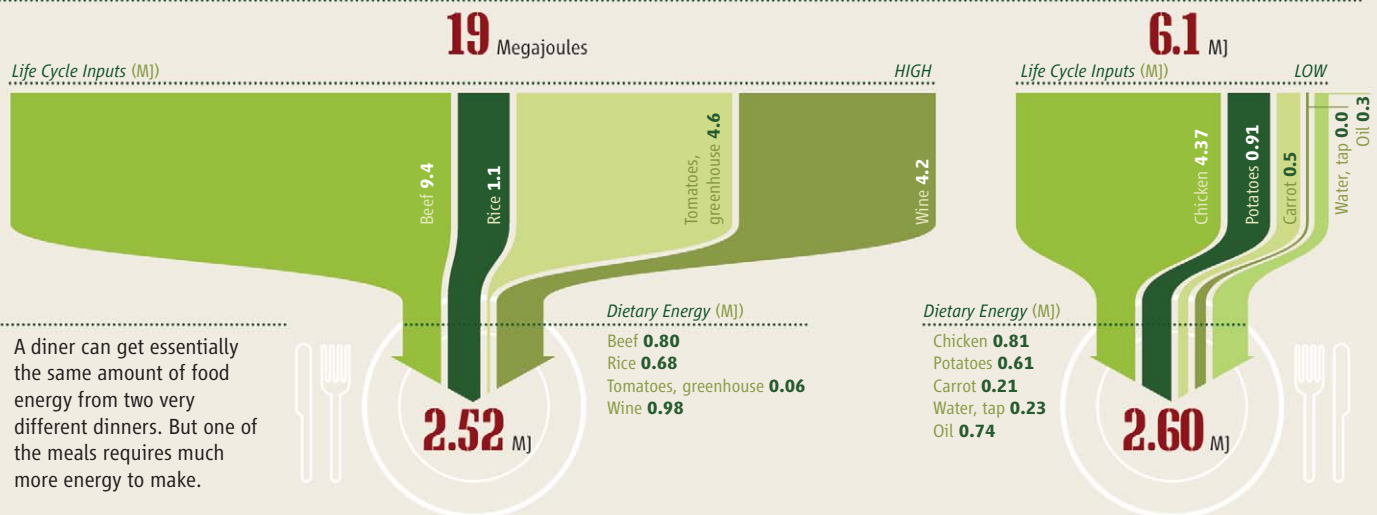
ALL BEANS AREN'T CREATED EQUAL

A Swedish study found the amount of energy needed to produce staple beans can vary widely depending on where they are grown, and how they are packaged, transported, and cooked.



SOURCE: Carlsson-Kanyama, A. et al., *Ecological Economics* 44, Issues 2–3 (March 2003)

TABLE FOR ONE: Which Is the Greener Plate?



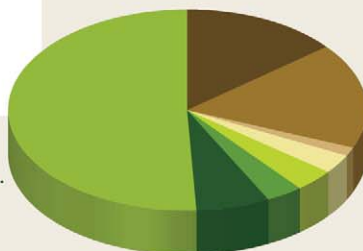
SOURCE: Carlsson-Kanyama, A. et al., *Ecological Economics* 44, Issues 2–3 (March 2003)

POWER FOOD: Energy Used for a Week's Meals

- Food supply 170 MJ/wk
- Primary packaging 25 MJ/wk
- Transport packaging 12 MJ/wk
- Transport from factory 12 MJ/wk
- Retailing 10 MJ/wk
- Travel to shops 5 MJ/wk
- Home storage 58 MJ/wk
- Home cooking 46 MJ/wk

In the United Kingdom, one study concluded that the amount of energy that goes into producing a week's supply of food is nearly five times greater than what the eater gets out of the final product.

338 MJ/person/week
Energy to produce and deliver food from field to fork

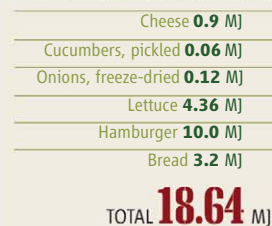


73 MJ/person/week
Energy that the average person gets in a week from all food

SOURCE: INCPEN UK

ENERGY BURGER

Beef is the most energy-intensive ingredient in a classic McDonald's hamburger, according to a Swedish study.



SOURCE: Carlsson-Kanyama, A./Dept. of Systems Ecology, Stockholm U.

NEWS

Could Less Meat Mean More Food?

HERE'S A SIMPLE IDEA YOU MAY HAVE HEARD FOR IMPROVING FOOD security: Eat less meat.

The logic—articulated by groups that include the Vegetarian Society of the United Kingdom and the United Nations Environment Programme—goes like this. From chicken cordon bleu to bacon double cheeseburgers, people in the developed world eat a huge amount of animal protein. And consumption of meat, eggs, and milk is already growing globally as people in poorer nations get richer and shift their diets. That's a problem because animals are eating a growing share of the world's grain harvests—and already directly or indirectly utilize up to 80% of the world's agricultural land. Yet they supply just 15% of all calories. So, the argument goes, if we just ate less meat, we could free up a lot of plants to feed billions of hungry people and gain a lot of good farmland.

Some food-security researchers, however, are skeptical. Although cutting back on meat has many potential benefits, they say the complexities of global markets and human food traditions could also produce some counterintuitive—and possibly counterproductive—results. “It's not this panacea that people have put forward,” says Mark Rosegrant of the International Food Policy Research Institute (IFPRI) in Washington, D.C. One provocative forecast: If people in industrialized nations gave up half their meat, more Asian children could become malnourished.

Protein-rich

Scholars on all sides of the meaty issue agree on one thing: Just as the rich use more energy than the poor, they also eat more meat. The United States, for instance, has just 4.5% of the world's population but accounts for about 15% of global meat consumption. Americans consume about 330 grams of meat a day on average—the equivalent of three quarter-pound hamburgers. In contrast, the U.S. Department of Agriculture recommends that most people consume just 142 to 184 grams of meat and beans daily. In the developing world, daily meat consumption averages just 80 grams.

Those numbers suggest that people living in the United States and other wealthy nations could increase world grain supplies simply by forgoing that extra burger or chop. But it's not that simple. Figuring out the full impact of meat consumption on global food security requires sophisticated computer models that can track how buying decisions ripple out across farming systems, global supply chains, and food markets.

One of those models is called IMPACT, and in 1998 IFPRI's Rosegrant and colleagues used it to study what might happen in 2020 if rich nations cut their per capita demand for meat to half of what it was in 1993. First, the simulation found that as demand for meat fell, prices declined and meat became more affordable worldwide. As a result, in the developing world, per capita meat consumption actually *increased* by 13% as poorer consumers could buy more. That's good news for what could be called “meat equity,” because increasing animal-protein consumption among the very



Grassroots. Livestock consume grain and resources that could be used to feed people. War rationing has inspired efforts to persuade people to eat less meat.



poor can provide substantial nutritional benefits, particularly for children.

Surprisingly, however, when the rich halved their meat habit, the poor didn't necessarily get that much more grain—their largest source of calories. According to the model, per capita cereal consumption in developing nations rose by just 1.5%. That's enough grain to ease hunger for 3.6 million malnourished children—but nowhere near the kinds of gains many expect from curbing meat consumption.

One big reason is the mismatch between human and animal diets. In rich countries, farmers usually feed their livestock corn or soybeans. When the farmers produce less meat, demand for corn and soy drops and the grains become more affordable. That's good for people in the parts of Africa and Latin America where corn is a dietary staple. But people in many developing countries, particularly in Asia, don't eat much corn; they eat rice and wheat. So falling corn and soy prices don't directly help them. (It's true that as demand for corn drops, some farmers might start growing wheat instead. In general, however, climate, soil, or water availability often limit a farmer's ability to switch crops easily. Iowa soybean growers, for instance, can't start growing rice, which requires heavy irrigation.)

Eating less meat could even backfire and make food insecurity worse, suggested the simulation, which was published in the *Proceedings of the Nutrition Society*. For instance, when consumers in developed countries replaced meat with pasta and bread, world wheat prices rose. That actually increased malnutrition slightly in developing countries such as India that rely on wheat. “It's a big deal when wheat prices go up,” Rosegrant says.

When all the pluses and minuses are added up, Rosegrant is confident that cutting meat consumption could ultimately help improve global food security. But “it's a small contribution, like changing to fluorescent light bulbs” to fight global warming, he says.

Changing appetites

Given the world's voracious and growing appetite for animal products, however, how could people be persuaded to eat less? One approach, scholars say, is to raise the price to reduce demand. If meat prices reflected the true ecological and climate costs of raising farm animals, for instance, many people would buy less, suggests Lester Brown of the Earth Policy Institute in Washington, D.C. He'd like to see taxes that are tied to meat's carbon footprint. Beef might get higher taxes than chicken or catfish, he says, predicting that such levies "would free up grain for those further down the food chain."

A similar approach calls for removing subsidies—both obvious and hidden—for meat producers. Beef exporter Brazil, for instance, indirectly subsidizes meat consumption by not charging consumers for the tropical forests destroyed by ranching, argues Sjur Kasa, a sociologist at the University of Oslo. Ending subsidies would be "the most powerful tool for curbing meat consumption," Kasa says, but it would be "a very difficult battle." So far, however, the battle hasn't been joined. "There are really no big victories when it comes to making people eat less meat for sustainability reasons," he says.

Campaigns directed at consumers, emphasizing the health benefits of reducing calories and animal fats, could prove a winner, says Danielle Nierenberg of the Worldwatch Institute in Washington, D.C. She notes that concerns about health care costs and a greater focus on preventing disease have helped spur a number of innovative efforts. In 2003, for instance, the Johns Hopkins Bloomberg School of Public Health started "Meatless Mondays," an initiative to reduce U.S. meat consumption by 15%. The organizers were inspired in part by government campaigns during World War I and II to ration meat for troops. In May 2009, the city council of Ghent, Belgium, proclaimed that its citizens should avoid eating meat on Thursdays. And last fall, Baltimore became the first city to serve only vegetarian meals 1 day a week in public schools.

So far, it's hard to know if these small-scale efforts have had any significant impact. And Rosegrant has an overarching concern: "What worries me is that people will think that's all we need to do." To truly ensure global food security, he says we'll also need much greater investment in agricultural research to boost yields and more economic development that increases incomes in poorer nations. "We have to go beyond personal responsibility," he says, "to policy action."

—ERIK STOKSTAD

NEWS

For More Protein, Filet of Cricket

COULD AN AFRICAN CATERPILLAR BE THE NEW BEEFSTEAK?

As the world diverts more of its grain harvests into producing meat, some scientists are pushing policymakers to take a closer look at insects as an environmentally friendlier source of protein. Whereas a cow needs to eat roughly 8 grams of food to gain a gram in weight, for instance, insects need less than two. "If you are going to feed 9 billion people, we cannot ignore the efficiency of insects as protein producers," says Paul Vantomme, senior forestry officer at the United Nations Food and Agriculture Organization (FAO) in Rome.

Consider, for instance, the mopane worm. These caterpillars of the emperor moth feed on the leaves of mopane (mo-PAN-ee) trees, which emerge in southern Africa's summer, a time when other staples can be in short supply. Dried, stewed, smoked, or fried, the insects are

a popular delicacy. And they are just one of hundreds of insect species that play an important role in the diets of millions of people.

"Nutritionally, it is excellent food," says Arnold van Huis, an entomologist at Wageningen University in the Netherlands. "It's the same or even better than conventional meat, fish, or poultry." Just 100 grams of caterpillars can provide all of an adult's recommended daily protein, along with iron, B vitamins, and other essential nutrients, he says.

Such eye-opening statistics have prompted FAO to develop new policy guidelines—expected later this year—that will encourage countries to include insects in their food-security plans. Vantomme hopes the guidelines will lead to more constructive discussions about managing insects. Currently, he says, "some [advisers] get their insecticides ready, and others get their chopsticks."

Currently, most edible insects are collected in the wild. In Mexico, for instance, farmers collect *chapulines* (young grasshoppers) from their maize and alfalfa fields, where they would otherwise do damage. FAO, however, is taking a closer look at experimental insect breeding to see whether it can be both ecologically and economically sustainable. Researchers are also studying whether they could use insect protein in livestock feed or even as a food additive.

A scattering of enthusiasts think that entomophagy—the technical term for eating insects—could even catch on among Europeans and North Americans. In the Netherlands, a company called Bugs Organic Food markets mealworms and grasshoppers through two dozen outlets. The effort has had some success—even "the minister of agriculture held a grasshopper" at a press conference, van Huis says. She didn't eat the hopper but did approve subsidies for Bugs Organic Food to further develop their products.

—GRETCHEN VOGEL



Crunchy delight.
Grasshoppers known as *chapulines* in a Mexican market.

CREDIT: KRISTEN KASTNER

REVIEW

Food Security: The Challenge of Feeding 9 Billion People

H. Charles J. Godfray,^{1*} John R. Beddington,² Ian R. Crute,³ Lawrence Haddad,⁴ David Lawrence,⁵ James F. Muir,⁶ Jules Pretty,⁷ Sherman Robinson,⁸ Sandy M. Thomas,⁹ Camilla Toulmin¹⁰

Continuing population and consumption growth will mean that the global demand for food will increase for at least another 40 years. Growing competition for land, water, and energy, in addition to the overexploitation of fisheries, will affect our ability to produce food, as will the urgent requirement to reduce the impact of the food system on the environment. The effects of climate change are a further threat. But the world can produce more food and can ensure that it is used more efficiently and equitably. A multifaceted and linked global strategy is needed to ensure sustainable and equitable food security, different components of which are explored here.

The past half-century has seen marked growth in food production, allowing for a dramatic decrease in the proportion of the world's people that are hungry, despite a doubling of the total population (Fig. 1) (1, 2). Nevertheless, more than one in seven people today still do not have access to sufficient protein and energy from their diet, and even more suffer from some form of micronutrient malnourishment (3). The world is now facing a new set of intersecting challenges (4). The global population will continue to grow, yet it is likely to plateau at some 9 billion people by roughly the middle of this century. A major correlate of this deceleration in population growth is increased wealth, and with higher purchasing power comes higher consumption and a greater demand for processed food, meat, dairy, and fish, all of which add pressure to the food supply system. At the same time, food producers are experiencing greater competition for land, water, and energy, and the need to curb the many negative effects of food production on the environment is becoming increasingly clear (5, 6). Overarching all of these issues is the threat of the effects of substantial climate change and concerns about how mitigation and adaptation measures may affect the food system (7, 8).

A threefold challenge now faces the world (9): Match the rapidly changing demand for food

from a larger and more affluent population to its supply; do so in ways that are environmentally and socially sustainable; and ensure that the world's poorest people are no longer hungry. This challenge requires changes in the way food is produced, stored, processed, distributed, and accessed that are as radical as those that occurred

during the 18th- and 19th-century Industrial and Agricultural Revolutions and the 20th-century Green Revolution. Increases in production will have an important part to play, but they will be constrained as never before by the finite resources provided by Earth's lands, oceans, and atmosphere (10).

Patterns in global food prices are indicators of trends in the availability of food, at least for those who can afford it and have access to world markets. Over the past century, gross food prices have generally fallen, leveling off in the past three decades but punctuated by price spikes such as that caused by the 1970s oil crisis. In mid-2008, there was an unexpected rapid rise in food prices, the cause of which is still being debated, that subsided when the world economy went into recession (11). However, many (but not all) commentators have predicted that this spike heralds a period of rising and more volatile food prices driven primarily by increased demand from rapidly developing countries, as well as by competition for resources from first-generation biofuels production (12). Increased food prices will stimulate greater investment in food production, but the critical importance of food to human well-being and also to social and political stability makes it likely that governments and other organizations will want to encourage food production beyond that driven by simple market mechanisms (13). The long-term nature of returns on investment for many aspects of food production and the importance of policies that promote sustainability and equity also argue against purely relying on market solutions.

So how can more food be produced sustainably? In the past, the primary solution to food shortages has been to bring more land into agriculture and to exploit new fish stocks. Yet over the past 5 decades, while grain production has more than doubled, the amount of land devoted to arable agriculture globally has increased by only ~9% (14). Some new land could be brought into cultivation, but the competition for land from other human activities makes this an increasingly unlikely and costly solution, particularly if protecting biodiversity and the public goods provided by natural ecosystems (for example, carbon storage in rainforest) are given higher priority (15). In recent decades, agricultural land that was formerly productive has been lost to urbanization and other human uses, as well as to desertification, salinization, soil erosion, and other consequences of unsustainable land

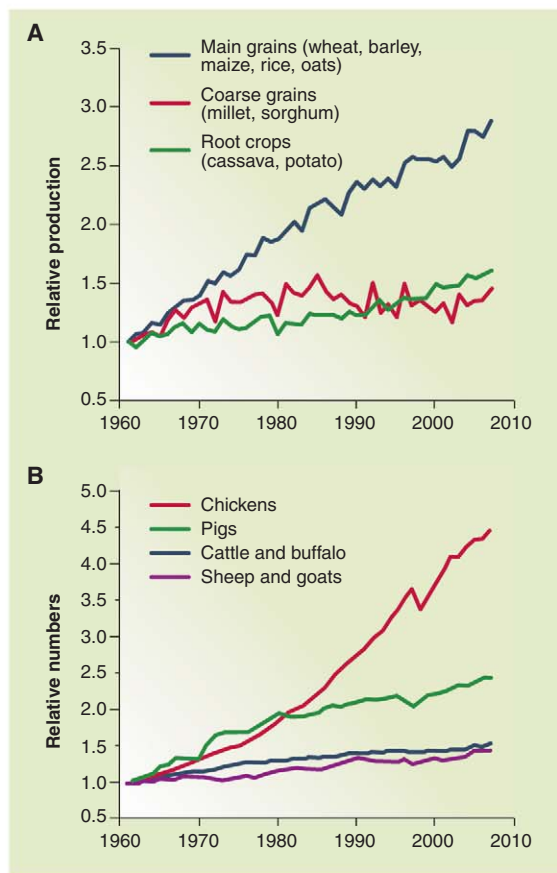


Fig. 1. Changes in the relative global production of crops and animals since 1961 (when relative production scaled to 1 in 1961). (A) Major crop plants and (B) major types of livestock. [Source: (2)]

¹Department of Zoology and Institute of Biodiversity at the James Martin 21st Century School, University of Oxford, South Parks Road, Oxford OX1 3PS, UK. ²U.K. Government Office for Science, 1 Victoria Street, London SW1H 0ET, UK. ³Agriculture and Horticulture Development Board, Stoneleigh Park, Kenilworth, Warwickshire CV8 2TL, UK. ⁴Institute of Development Studies, Falmer, Brighton BN1 9RE, UK. ⁵Syngenta AG, Post Office Box, CH-4002 Basel, Switzerland. ⁶Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, UK. ⁷Department of Biological Sciences, University of Essex, Wivenhoe Park, Colchester, Essex CO4 3SQ, UK. ⁸Institute of Development Studies, Falmer, Brighton BN1 9RE, UK. ⁹Foresight, U.K. Government Office for Science, 1 Victoria Street, London SW1H 0ET, UK. ¹⁰International Institute for Environment and Development, 3 Endsleigh Street, London WC1H 0DD, UK.

*To whom correspondence should be addressed. E-mail: charles.godfray@zoo.ox.ac.uk

management (16). Further losses, which may be exacerbated by climate change, are likely (7). Recent policy decisions to produce first-generation biofuels on good quality agricultural land have added to the competitive pressures (17). Thus, the most likely scenario is that more food will need to be produced from the same amount of (or even less) land. Moreover, there are no major new fishing grounds: Virtually all capture fisheries are fully exploited, and most are overexploited.

Recent studies suggest that the world will need 70 to 100% more food by 2050 (1, 18). In this article, major strategies for contributing to the challenge of feeding 9 billion people, including the most disadvantaged, are explored. Particular emphasis is given to sustainability, as well as to the combined role of the natural and social sciences in analyzing and addressing the challenge.

Closing the Yield Gap

There is wide geographic variation in crop and livestock productivity, even across regions that experience similar climates. The difference between realized productivity and the best that can be achieved using current genetic material and available technologies and management is termed the “yield gap.” The best yields that can be obtained locally depend on the capacity of farmers to access and use, among other things, seeds, water, nutrients, pest management, soils, biodiversity, and knowledge. It has been estimated that in those parts of Southeast Asia where irrigation is available, average maximum climate-adjusted rice yields are 8.5 metric tons per hectare, yet the average actually achieved yields are 60% of this figure (19). Similar yield gaps are found in rain-fed wheat in central Asia and rain-fed cereals in Argentina and Brazil. Another way to illustrate the yield gap is to compare changes in per capita food production over the past 50 years. In Asia, this amount has increased approximately twofold (in China, by a factor of nearly 3.5), and in Latin America, it has increased 1.6-fold; in Africa, per capita production fell back from the mid-1970s and has only just reached the same level as in 1961 (2, 20). Substantially more food, as well as the income to purchase food, could be produced with current crops and livestock if methods were found to close the yield gaps.

Low yields occur because of technical constraints that prevent local food producers from

increasing productivity or for economic reasons arising from market conditions. For example, farmers may not have access to the technical knowledge and skills required to increase production, the finances required to invest in higher production (e.g., irrigation, fertilizer, machinery, crop-protection products, and soil-conservation measures), or the crop and livestock varieties that maximize yields. After harvest or slaughter, they may not be able to store the produce or have access to the infrastructure to transport the produce to consumer markets. Farmers may also choose not to invest in improving agricultural

Box 1. Sustainable intensification.

Producing more food from the same area of land while reducing the environmental impacts requires what has been called “sustainable intensification” (18). In exactly the same way that yields can be increased with the use of existing technologies, many options currently exist to reduce negative externalities (47). Net reductions in some greenhouse gas emissions can potentially be achieved by changing agronomic practices, the adoption of integrated pest management methods, the integrated management of waste in livestock production, and the use of agroforestry. However, the effects of different agronomic practices on the full range of greenhouse gases can be very complex and may depend on the temporal and spatial scale of measurement. More research is required to allow a better assessment of competing policy options. Strategies such as zero or reduced tillage (the reduction in inversion ploughing), contour farming, mulches, and cover crops improve water and soil conservation, but they may not increase stocks of soil carbon or reduce emissions of nitrous oxide. Precision agriculture refers to a series of technologies that allow the application of water, nutrients, and pesticides only to the places and at the times they are required, thereby optimizing the use of inputs (48). Finally, agricultural land and water bodies used for aquaculture and fisheries can be managed in ways specifically designed to reduce negative impacts on biodiversity.

productivity because the returns do not compare well with other uses of capital and labor.

Exactly how best to facilitate increased food production is highly site-specific. In the most extreme cases of failed states and nonfunctioning markets, the solution lies completely outside the food system. Where a functioning state exists, there is a balance to be struck between investing in overall economic growth as a spur to agriculture and focusing on investing in agriculture as a spur to economic growth, though the two are obviously linked in regions, such as sub-Saharan Africa, where agriculture typically makes up 20 to 40% gross domestic product. In some situations, such as low-income food-importing countries, investing purely in generating widespread income growth to allow food purchases from regions and countries with better production capabilities may be the best choice. When investment is targeted at food production, a further issue is the balance between putting resources into regional and national infrastructure, such as roads and ports, and investing in local social and economic capital (21, 22).

A yield gap may also exist because the high costs of inputs or the low returns from increased production make it economically suboptimal to raise production to the maximum technically attainable. Poor transport and market infrastructure raise the prices of inputs, such as fertilizers and water, and increase the costs of moving the food produced into national or world markets. Where the risks of investment are high and the means to offset them are absent, not investing can be the most rational decision, part of the “poverty trap.” Food production in developing countries can be severely affected by market interventions in the developed world, such as subsidies or price supports. These need to be carefully designed and implemented so that their effects on global commodity prices do not act as disincentives to production in other countries (23).

The globalization of the food system offers some local food producers access to larger markets, as well as to capital for investment. At the aggregate level, it also appears to increase the global efficiency of food production by allowing regional specialization in the production of the locally most appropriate foods. Because the expansion of food production and the growth of population both occur at different rates in different geographic regions, global trade is necessary to balance supply and demand across regions. However, the environmental costs of food production might increase with globalization, for example, because of increased greenhouse gas emissions associated with increased production and food transport (24). An unfettered market can also penalize particular communities and sectors, especially the poorest who have the least influence on how global markets are structured and regulated. Expanded trade can provide insurance against regional shocks on production such as conflict, epidemics, droughts, or floods—shocks that are likely to increase in frequency as climate change occurs. Conversely, a highly connected food system may lead to the more widespread propagation of economic perturbations, as in the recent banking crisis, thus affecting more people. There is an urgent need for a better understanding of the effects of globalization on the full food system and its externalities.

The yield gap is not static. Maintaining, let alone increasing, productivity depends on continued innovation to control weeds, diseases, insects, and other pests as they evolve resistance to different control measures, or as new species emerge or are dispersed to new regions.

Innovation involves both traditional and advanced crop and livestock breeding, as well as the continuing development of better chemical, agronomic, and agro-ecological control measures. The maximum attainable yield in different regions will also shift as the effects of climate change are felt. Increasing atmospheric CO₂ levels can directly stimulate crop growth, though within the context of real agricultural production systems, the magnitude of this effect is not clear (7). More important will be the ability to grow crops in places that are currently unsuitable, particularly the northern temperate regions (though expansion of agriculture at the expense of boreal forest would lead to major greenhouse gas emissions), and the loss of currently productive regions because of excessively high temperatures and drought. Models that couple the physics of climate change with the biology of crop growth will be important to help policy-makers anticipate these changes, as well as to evaluate the role of “agricultural biodiversity” in helping mitigate their effects (25).

Closing the yield gap would dramatically increase the supply of food, but with uncertain impacts on the environment and potential feedbacks that could undermine future food production. Food production has important negative “externalities,” namely effects on the environment or economy that are not reflected in the cost of food. These include the release of greenhouse gases [especially methane and nitrous oxide, which are more damaging than CO₂ and for which agriculture is a major source (26)], environmental pollution due to nutrient run-off, water shortages due to overextraction, soil degradation and the loss of biodiversity through land conversion or inappropriate management, and ecosystem disruption due to the intensive harvesting of fish and other aquatic foods (6).

To address these negative effects, it is now widely recognized that food production systems and the food chain in general must become fully sustainable (18). The principle of sustainability implies the use of resources at rates that do not exceed the capacity of Earth to replace them. By definition, dependency on nonrenewable inputs is unsustainable, even if in the short term it is necessary as part of a trajectory toward sustainability.

There are many difficulties in making sustainability operational. Over what spatial scale should food production be sustainable? Clearly an overarching goal is global sustainability, but should this goal also apply at lower levels, such as regions (or oceans), nations, or farms? Could high levels of consumption or negative externalities in some regions be mitigated by improvements in other areas, or could some unsustainable activities in the food system be offset by actions in the nonfood sector (through carbon-trading, for example)? Though simple definitions of sustainability are independent of time scale, in

practice, how fast should we seek to move from the status quo to a sustainable food system? The challenges of climate change and competition for water, fossil fuels, and other resources suggest that a rapid transition is essential. Nevertheless, it is also legitimate to explore the possibility that superior technologies may become available and that future generations may be wealthier and, hence, better able to absorb the costs of the transition. Finally, we do not yet have good enough

search on the ability of these and related programs to be scaled up to country and regional levels should be a priority (Fig. 2).

Strategies designed to close the yield gap in the poorest countries face some particular challenges (28). Much production is dominated by small-holder agriculture with women often taking a dominant role in the workforce. Where viable, investment in the social and economic mechanisms to enable improved small-holder yields,

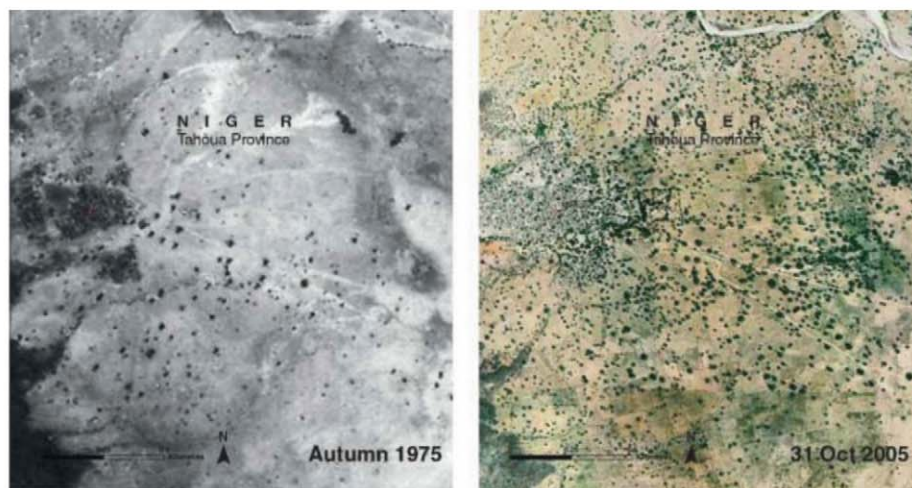


Fig. 2. An example of a major successful sustainable agriculture project. Niger was strongly affected by a series of drought years in the 1970s and 1980s and by environmental degradation. From the early 1980s, donors invested substantially in soil and water conservation. The total area treated is on the order of 300,000 ha, most of which went into the rehabilitation of degraded land. The project in the Illela district of Niger promoted simple water-harvesting techniques. Contour stone bunds, half moons, stone bunding, and improved traditional planting pits (zai) were used to rehabilitate barren, crusted land. More than 300,000 ha have been rehabilitated, and crop yields have increased and become more stable from year to year. Tree cover has increased, as shown in the photographs. Development of the land market and continued incremental expansion of the treated area without further project assistance indicate that the outcomes are sustainable (51, 52).

metrics of sustainability, a major problem when evaluating alternative strategies and negotiating trade-offs. This is the case for relatively circumscribed activities, such as crop production on individual farms, and even harder when the complete food chain is included or for complex products that may contain ingredients sourced from all around the globe. There is also a danger that an overemphasis on what can be measured relatively simply (carbon, for example) may lead to dimensions of sustainability that are harder to quantify (such as biodiversity) being ignored. These are areas at the interface of science, engineering, and economics that urgently need more attention (see Box 1). The introduction of measures to promote sustainability does not necessarily reduce yields or profits. One study of 286 agricultural sustainability projects in developing countries, involving 12.6 million chiefly small-holder farmers on 37 million hectares, found an average yield increase of 79% across a very wide variety of systems and crop types (27). One-quarter of the projects reported a doubling of yield. Re-

especially where targeted at women, can be important means of increasing the income of both farm and rural nonfarm households. The lack of secure land rights can be a particular problem for many poor communities, may act as a disincentive for small holders to invest in managing the land more productively, and may make it harder to raise investment capital (29). In a time of rising prices for food and land, it can also render these communities vulnerable to displacement by more powerful interest groups. Where the political will and organizational infrastructure exist, title definition and protection could be greatly assisted by the application of modern information and communication technologies. Even so, there will be many people who cannot afford to purchase sufficient calories and nutrients for a healthy life and who will require social protection programs to increase their ability to obtain food. However, if properly designed, these programs can help stimulate local agriculture by providing small holders with increased certainty about the demand for their products.

There is also a role for large-scale farming operations in poor-country agriculture, though the value and contexts in which this is feasible are much debated (30). This debate has been fanned by a substantial increase in the number of sovereign wealth funds, companies, and individuals leasing, purchasing, or attempting to purchase large tracts of agricultural land in developing countries. This external investment in developing-country agriculture may bring major benefits, especially where investors bring considerable improvements to crop production and processing, but only if the rights and welfare of the tenants and existing resource users are properly addressed (31).

Many of the very poorest people live in areas so remote that they are effectively disconnected from national and world food markets. But for others, especially the urban poor, higher food prices have a direct negative effect on their ability to purchase a healthy diet. Many rural farmers and other food producers live near the margin of being net food consumers and producers and will be affected in complex ways by rising food prices, with some benefitting and some being harmed (21). Thus, whereas reducing distorting agricultural support mechanisms in developed countries and liberalizing world trade should stimulate overall food production in developing countries, not everyone will gain (23, 32). Better models that can more accurately predict these complex interactions are urgently needed.

Increasing Production Limits

The most productive crops, such as sugar cane, growing in optimum conditions, can convert solar energy into biomass with an efficiency of ~2%, resulting in high yields of biomass (up to 150 metric tons per hectare) (33). There is much debate over exactly what the theoretical limits are for the major crops under different conditions, and similarly, for the maximum yield that can be obtained for livestock rearing (18). However, there is clearly considerable scope for increasing production limits.

The Green Revolution succeeded by using conventional breeding to develop F₁ hybrid varieties of maize and semi-dwarf, disease-resistant varieties of wheat and rice. These varieties could be provided with more irrigation and fertilizer (20) without the risk of major crop losses due to lodging (falling over) or severe rust epidemics. Increased yield is still a major goal, but the importance of greater water- and nutrient-use efficiency, as well as tolerance of abiotic stress, is also likely to increase. Modern genetic techniques and a better understanding of crop physiology allow for a more directed approach to selection across multiple traits. The speed and costs at which genomes today can be sequenced or resequenced now means that these techniques can be more easily applied to develop varieties of crop species that will yield well in challenging environments.

Table 1. Examples of current and potential future applications of GM technology for crop genetic improvement. [Source: (18, 49)]

Time scale	Target crop trait	Target crops
Current	Tolerance to broad-spectrum herbicide	Maize, soybean, oilseed brassica
	Resistance to chewing insect pests	Maize, cotton, oilseed brassica
Short-term (5–10 years)	Nutritional bio-fortification	Staple cereal crops, sweet potato
	Resistance to fungus and virus pathogens	Potato, wheat, rice, banana, fruits, vegetables
	Resistance to sucking insect pests	Rice, fruits, vegetables
	Improved processing and storage	Wheat, potato, fruits, vegetables
Medium-term (10–20 years)	Drought tolerance	Staple cereal and tuber crops
	Salinity tolerance	Staple cereal and tuber crops
	Increased nitrogen-use efficiency	
Long-term (>20 years)	High-temperature tolerance	
	apomixis	Staple cereal and tuber crops
	Nitrogen fixation	
	Denitrification inhibitor production	
	Conversion to perennial habit	
	Increased photosynthetic efficiency	

These include crops such as sorghum, millet, cassava, and banana, species that are staple foods for many of the world's poorest communities (34).

Currently, the major commercialized genetically modified (GM) crops involve relatively simple manipulations, such as the insertion of a gene for herbicide resistance or another for a pest-insect toxin. The next decade will see the development of combinations of desirable traits and the introduction of new traits such as drought tolerance. By mid-century, much more radical options involving highly polygenic traits may be feasible (Table 1). Production of cloned animals with engineered innate immunity to diseases that reduce production efficiency has the potential to reduce substantial losses arising from mortality and subclinical infections. Biotechnology could also produce plants for animal feed with modified composition that increase the efficiency of meat production and lower methane emissions.

Domestication inevitably means that only a subset of the genes available in the wild-species progenitor gene pool is represented among crop varieties and livestock breeds. Unexploited genetic material from land races, rare breeds, and wild relatives will be important in allowing breeders to respond to new challenges. International collections and gene banks provide valuable repositories for such genetic variation, but it is nevertheless necessary to ensure that locally adapted crop and livestock germplasm is not lost in the process of their displacement by modern, improved varieties and breeds. The trend over recent decades is of a general decline in investment in technological innovation in food produc-

tion (with some notable exceptions, such as in China and Brazil) and a switch from public to private sources (1). Fair returns on investment are essential for the proper functioning of the private sector, but the extension of the protection of intellectual property rights to biotechnology has led to a growing public perception in some countries that biotech research purely benefits commercial interests and offers no long-term public good. Just as seriously, it also led to a virtual monopoly of GM traits in some parts of the world, by a restricted number of companies, which limits innovation and investment in the technology. Finding ways to incentivize wide access and sustainability, while encouraging a competitive and innovative private sector to make best use of developing technology, is a major governance challenge.

The issue of trust and public acceptance of biotechnology has been highlighted by the debate over the acceptance of GM technologies. Because genetic modification involves germline modification of an organism and its introduction to the environment and food chain, a number of particular environmental and food safety issues need to be assessed. Despite the introduction of rigorous science-based risk assessment, this discussion has become highly politicized and polarized in some countries, particularly those in Europe. Our view is that genetic modification is a potentially valuable technology whose advantages and disadvantages need to be considered rigorously on an evidential, inclusive, case-by-case basis: Genetic modification should neither be privileged nor automatically dismissed. We also accept the

need for this technology to gain greater public acceptance and trust before it can be considered as one among a set of technologies that may contribute to improved global food security.

There are particular issues involving new technologies, both GM and non-GM, that are targeted at helping the least-developed countries (35, 36). The technologies must be directed at the needs of those communities, which are often different from those of more developed country farmers. To increase the likelihood that new technology works for, and is adopted by, the poorest nations, they need to be involved in the framing, prioritization, risk assessment, and regulation of innovations. This will often require the creation of innovative institutional and governance mechanisms that account for socio-cultural context (for example, the importance of women in developing-country food production). New technologies offer major promise, but there are risks of lost trust if their potential benefits are exaggerated in public debate. Efforts to increase sustainable production limits that benefit the poorest nations will need to be based around new alliances of businesses, civil society organizations, and governments.

Reducing Waste

Roughly 30 to 40% of food in both the developed and developing worlds is lost to waste, though the causes behind this are very different (Fig. 3) (16, 37–39). In the developing world, losses are mainly attributable to the absence of food-chain infrastructure and the lack of knowledge or investment in storage technologies on the farm, although data are scarce. For example, in India, it is estimated that 35 to 40% of fresh produce is lost because neither wholesale nor retail outlets have cold storage (16). Even with rice grain, which can be stored more readily, as much as one-third of the harvest in Southeast Asia can be lost after harvest to pests and spoilage (40). But the picture is more complex than a simple lack of storage facilities: Although storage after harvest when there is a glut of food would seem to make economic sense, the farmer often has to sell immediately to raise cash.

In contrast, in the developed world, pre-retail losses are much lower, but those arising at the retail, food service, and home stages of the food chain have grown dramatically in recent years, for a variety of reasons (41). At present, food is relatively cheap, at least for these consumers, which reduces the incentives to avoid waste. Consumers have become accustomed to purchasing foods of the highest cosmetic standards; hence, retailers discard many edible, yet only slightly

blemished products. Commercial pressures can encourage waste: The food service industry frequently uses “super-sized” portions as a competitive lever, whereas “buy one get one free” offers have the same function for retailers. Litigation and lack of education on food safety have led to a reliance on “use by” dates, whose safety margins often mean that food fit for consumption is thrown away. In some developed countries, unwanted food goes to a landfill instead of being used as animal feed or compost because of legislation to control prion diseases.

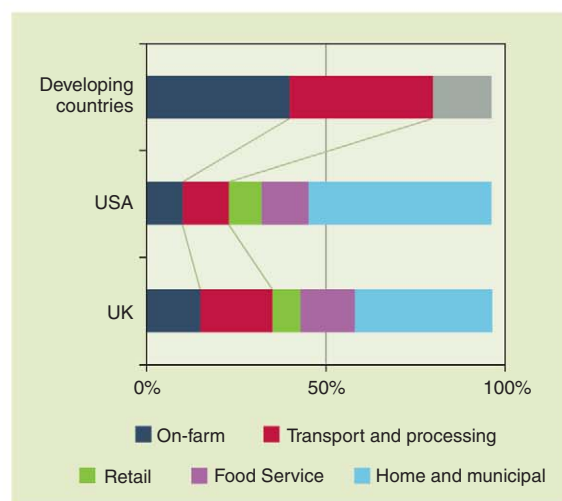


Fig. 3. Makeup of total food waste in developed and developing countries. Retail, food service, and home and municipal categories are lumped together for developing countries. [Source: (16, 37–39)]

Different strategies are required to tackle the two types of waste. In developing countries, public investment in transport infrastructure would reduce the opportunities for spoilage, whereas better-functioning markets and the availability of capital would increase the efficiency of the food chain, for example, by allowing the introduction of cold storage (though this has implications for greenhouse gas emissions) (38). Existing technologies and best practices need to be spread by education and extension services, and market and finance mechanisms are required to protect farmers from having to sell at peak supply, leading to gluts and wastage. There is also a need for continuing research in postharvest storage technologies. Improved technology for small-scale food storage in poorer contexts is a prime candidate for the introduction of state incentives for private innovation, with the involvement of small-scale traders, millers, and producers.

If food prices were to rise again, it is likely that there would be a decrease in the volume of waste produced by consumers in developed countries. Waste may also be reduced by alerting consumers to the scale of the issue, as well as to domestic strategies for reducing food loss. Ad-

vocacy, education, and possibly legislation may also reduce waste in the food service and retail sectors. Legislation such as that on sell-by dates and swill that has inadvertently increased food waste should be reexamined within a more inclusive competing-risks framework. Reducing developed-country food waste is particularly challenging, as it is so closely linked to individual behavior and cultural attitudes toward food.

Changing Diets

The conversion efficiency of plant into animal matter is ~10%; thus, there is a *prima facie* case that more people could be supported from the same amount of land if they were vegetarians. About one-third of global cereal production is fed to animals (42). But currently, one of the major challenges to the food system is the rapidly increasing demand for meat and dairy products that has led, over the past 50 years, to a ~1.5-fold increase in the global numbers of cattle, sheep, and goats, with equivalent increases of ~2.5- and ~4.5-fold for pigs and chickens, respectively (2) (Fig. 1). This is largely attributable to the increased wealth of consumers everywhere and most recently in countries such as China and India.

However, the argument that all meat consumption is bad is overly simplistic. First, there is substantial variation in the production efficiency and environmental impact of the major classes of meat consumed by people (Table 2). Second, although a substantial fraction of livestock is fed on grain and other plant protein that could feed humans, there remains a very substantial proportion that is grass-fed. Much of the grassland that is used to feed these animals could not be converted to arable land or could only be converted with majorly adverse environmental outcomes. In addition, pigs and poultry are often fed on human food “waste.” Third, through better rearing or improved breeds, it may be possible to increase the efficiency with which meat is produced. Finally, in developing countries, meat represents the most concentrated source of some vitamins and minerals, which is important for individuals such as young children. Livestock also are used for ploughing and transport, provide a local supply of manure, can be a vital source of income, and are of huge cultural importance for many poorer communities.

Reducing the consumption of meat and increasing the proportion that is derived from the most efficient sources offer an opportunity to feed more people and also present other advantages (37). Well-balanced diets rich in grains and other vegetable products are considered to be more healthful than those containing a high proportion of meat (especially red meat) and dairy products. As developing countries consume more meat in combination with high-sugar and -fat foods, they may find themselves having to deal with obesity before they have overcome undernutrition, leading to an increase in spending on health that could

Table 2. Comparison of the impact of grazing and intensive (confined/industrialized) grain-fed livestock systems on water use, grain requirement, and methane production. Service water is that required for cleaning and washing livestock housing and other facilities. Dashes indicate combinations for which no data are available (either because it cannot be measured or because the combination does not exist). This table does not include other impacts of differing livestock management systems such as (i) nutrient run-off and pollution to surface and groundwater, (ii) protozoan and bacterial contamination of water and food, (iii) antibiotic residues in water and food, (iv) heavy metal from feed in soils and water, (v) odor nuisance from wastes, (vi) inputs used for feed production and lost to the environment, (vii) livestock-related land-use change. [Source: (7, 50)]

Water	Measure of water use	Grazing	Intensive
		Liters day ⁻¹ per animal at 15°C	
Cattle	Drinking water: all	22	103
	Service water: beef	5	11
	Service water: dairy	5	22
Pigs (lactating adult)	Drinking water	17	17
	Service water	25	125
Sheep (lactating adult)	Drinking water	9	9
	Service water	5	5
Chicken (broiler and layer)	Drinking water	1.3–1.8	1.3–1.8
	Service water	0.09–0.15	0.09–0.15
<i>Feed required to produce 1 kg of meat</i>		<i>kg of cereal per animal</i>	
Cattle		—	8
Pigs		—	4
Chicken (broiler)		—	1
<i>Methane emissions from cattle</i>		<i>kg of CH₄ per animal year⁻¹</i>	
Cattle: dairy (U.S., Europe)		—	117–128
Cattle: beef, dairy (U.S., Europe)		53–60	—
Cattle: dairy (Africa, India)		—	45–58
Cattle: grazing (Africa, India)		27–31	—

otherwise be used to alleviate poverty. Livestock production is also a major source of methane, a very powerful greenhouse gas, though this can be partially offset by the use of animal manure to replace synthetic nitrogen fertilizer (43). Of the five strategies we discuss here, assessing the value of decreasing the fraction of meat in our diets is the most difficult and needs to be better understood.

Expanding Aquaculture

Aquatic products (mainly fish, aquatic molluscs, and crustaceans) have a critical role in the food system, providing nearly 3 billion people with at least 15% of their animal protein intake (44).

In many regions, aquaculture has been sufficiently profitable to permit strong growth; replicating this growth in areas such as Africa where it has not occurred could bring major benefits. Technical advances in hatchery systems, feeds and feed-delivery systems, and disease management could all increase output. Future gains may also come from better stock selection, larger-scale production technologies, aquaculture in open seas and larger inland water bodies, and the culture of a wider range of species. The long production cycle of many species (typically 6 to 24 months) requires a financing system that is capable of providing working capital as well as offsetting risk. Wider production options (such as temperature and salinity tolerance and disease resistance) and cheaper feed substrates (for in-

stance, plant material with enhanced nutritional features) might also be accessed with the use of GM technologies.

Aquaculture may cause harm to the environment because of the release into water bodies of organic effluents or disease treatment chemicals, indirectly through its dependence on industrial fisheries to supply feeds, and by acting as a source of diseases or genetic contamination for wild species. Efforts to reduce these negative externalities and increase the efficiency of resource use [such as the fish in-to-fish out ratio (45)] have been spurred by the rise of sustainability certification programs, though these mainly affect only higher-value sectors. Gains in sustainability could come from concentrating on lower-trophic level species and in integrating aquatic and terrestrial food production, for example, by using waste from the land as food and nutrients. It will also be important to take a more strategic approach to site location and capacity within catchment or coastal zone management units (46).

Conclusions

There is no simple solution to sustainably feeding 9 billion people, especially as many become increasingly better off and converge on rich-country consumption patterns. A broad range of options, including those we have discussed here, needs to be pursued simultaneously. We are hopeful about scientific and technological inno-

vation in the food system, but not as an excuse to delay difficult decisions today.

Any optimism must be tempered by the enormous challenges of making food production sustainable while controlling greenhouse gas emission and conserving dwindling water supplies, as well as meeting the Millennium Development Goal of ending hunger. Moreover, we must avoid the temptation to further sacrifice Earth's already hugely depleted biodiversity for easy gains in food production, not only because biodiversity provides many of the public goods on which mankind relies but also because we do not have the right to deprive future generations of its economic and cultural benefits. Together, these challenges amount to a perfect storm.

Navigating the storm will require a revolution in the social and natural sciences concerned with food production, as well as a breaking down of barriers between fields. The goal is no longer simply to maximize productivity, but to optimize across a far more complex landscape of production, environmental, and social justice outcomes.

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REVIEW

Breeding Technologies to Increase Crop Production in a Changing World

Mark Tester* and Peter Langridge

To feed the several billion people living on this planet, the production of high-quality food must increase with reduced inputs, but this accomplishment will be particularly challenging in the face of global environmental change. Plant breeders need to focus on traits with the greatest potential to increase yield. Hence, new technologies must be developed to accelerate breeding through improving genotyping and phenotyping methods and by increasing the available genetic diversity in breeding germplasm. The most gain will come from delivering these technologies in developing countries, but the technologies will have to be economically accessible and readily disseminated. Crop improvement through breeding brings immense value relative to investment and offers an effective approach to improving food security.

Although more food is needed for the rapidly growing human population, food quality also needs to be improved, particularly for increased nutrient content. In addition, agricultural inputs must be reduced, especially those of nitrogenous fertilizers, if we are to reduce environmental degradation caused by emissions of CO₂ and nitrogenous compounds from agricultural processes. Furthermore, there are now concerns about our ability to increase or even sustain crop yield and quality in the face of dynamic environmental and biotic threats that will be particularly challenging in the face of rapid global environmental change. The current di-

version of substantial quantities of food into the production of biofuels puts further pressure on world food supplies (1).

Breeding and agronomic improvements have, on average, achieved a linear increase in food production globally, at an average rate of 32 million metric tons per year (2) (Fig. 1). However, to meet the recent Declaration of the World Summit on Food Security (3) target of 70% more food by 2050, an average annual increase in production of 44 million metric tons per year is required (Fig. 1), representing a 38% increase over historical increases in production, to be sustained for 40 years. This scale of sustained increase in global food production is unprecedented and requires substantial changes in methods for agronomic processes and crop improvement. Achieving this increase in food production in a stable environment would be challenging, but is undoubtedly much

more so given the additional pressures created by global environmental changes.

Global Environmental Change Alters Breeding Targets

Certain aspects of global environmental change are beneficial to agriculture. Rising CO₂ acts as a fertilizer for C3 crops and is estimated to account for approximately 0.3% of the observed 1% rise in global wheat production (4), although this benefit is likely to diminish, because rising temperatures will increase photorespiration and nighttime respiration. A benefit of rising temperatures is the alleviation of low-temperature inhibition of growth, which is a widespread limitation at higher latitudes and altitudes. Offsetting these benefits, however, are obvious deleterious changes, such as an increased frequency of damaging high-temperature events, new pest and disease pressures, and altered patterns of drought. Negative effects of other pollutants, notably ozone, will also reduce benefits to plant growth from rising CO₂ and temperature.

Particularly challenging for society will be changes in weather patterns that will require alterations in farming practices and infrastructure; for example, water storage and transport networks. Because one-third of the world's food is produced on irrigated land (5, 6), the likely impacts on global food production are many. Along with agronomic- and management-based approaches to improving food production, improvements in a crop's ability to maintain yields with lower water supply and quality will be critical. Put simply, we need to increase the tolerance of crops to drought and salinity.

In the context of global environmental change, the efficiency of nitrogen use has also emerged as a key target. Human activity has already more than doubled the amount of atmospheric N₂ fixed

Australian Centre for Plant Functional Genomics, University of Adelaide, South Australia SA 5064, Australia.

*To whom correspondence should be addressed. E-mail: mark.tester@acpfg.com.au

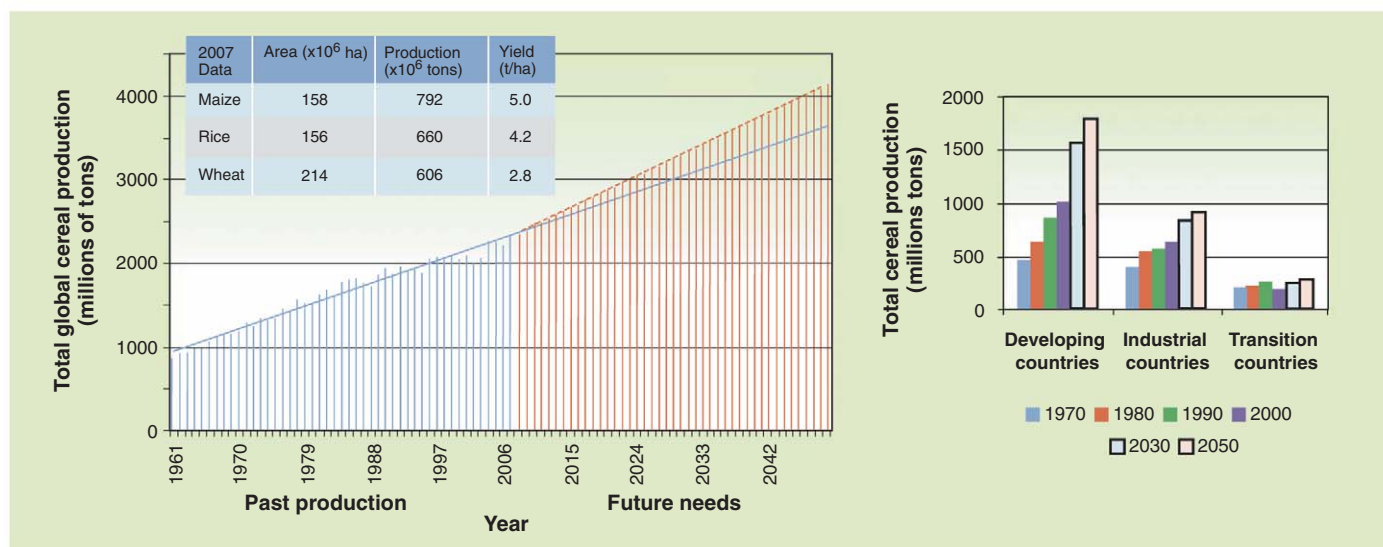


Fig. 1. Cereal production targets. (Left) Global cereal production has risen from 877 million metric tons in 1961 to 2351 million metric tons in 2007 (blue). However, to meet predicted demands (3), production will need to rise to over 4000 million metric tons by 2050 (red). The rate of yield increase must move from the blue trend line (32 million metric tons

per year) to the red dotted line (44 million metric tons per year) to meet this demand, an increase of 37%. The inset table shows the 2007 data for the three major cereals. Data are from the FAO: <http://faostat.fao.org/>. (Right) The greatest demand for yield increases will be from countries in the developing world. [Based on FAO data (26)].

annually, which has led to environmental impacts, such as increased water pollution, and the emission of greenhouse gases, such as nitrous oxide. Nitrogen inputs are increasingly being managed by legislation that limits fertilizer use in agriculture. Furthermore, rising energy costs means that fertilizers are now commonly the highest input cost for farmers. New crop varieties will need to be more efficient in their use of reduced nitrogen than current varieties are (7). Therefore, it is important that breeding programs develop strategies to select for yield and quality with lower nitrogen inputs.

Current Approaches to Crop Improvement

Arguably, increased yield in conditions of abiotic stresses, such as drought and salinity, could be best achieved by selecting for increased yield under optimal production conditions: Plants with higher yields in good conditions are more likely to have higher yields in stressed conditions (8). Such an approach will also increase yield in high-yield environments. However, it is becoming increasingly apparent that specific selection strategies are needed to enhance yield in low-yield (stressed) environments. Given that average global yields of wheat are less than 3 metric tons/ha (Fig. 1) and given there are many areas with yields as high as 10 metric tons/ha, the majority of land cropped to wheat delivers yields below 3 metric tons/ha. Therefore, by virtue of the much larger areas of low-yielding land globally, low-yielding environments offer the greatest opportunity for substantial increases in global food production. Increasing yield by 1 metric ton/ha in a low-yielding area delivers a much higher relative increase than does the same increase in

high-yielding environments. This increase can be achieved by tackling major limitations on yield in poor environments (termed yield stability); for example, by protecting plants and yield from factors such as salinity and heat or drought periods. The local social benefits of supporting farmers on low-yielding lands would also be great.

It is often thought that concentration on yield stability may come at the expense of high yields in good years; however, yield penalties in more favorable conditions do not necessarily accompany drought tolerance (Fig. 2). Yield stability is harder to select for than improved yield is, because selection in breeding programs requires many years and many sites for evaluation. However, there is evidence for a genetic basis for yield stability and, hence, an opportunity for gain (9). Transgenic approaches are also likely to improve yield stability (10). There are several clear examples where single genes have been able to substantially increase yield, notably to drive domestication (to control tiller number, branching, and seed number) and the green revolution (for dwarfing). Initial results suggest that a gene conferring increased drought tolerance may also have a widespread impact on yield (10).

This is not to say that efforts to maintain yield should be re-

duced. In particular, maintaining resistance to rapidly evolving pests and pathogens is an essential mainstay of breeding programs. Interactions between breeders, pathologists, and agronomists must be maintained to ensure that crops and cropping systems change coordinately. No-till farming, in which plowing of the soil is avoided, for example, has changed the spectrum of diseases and pests attacking crops, to the extent that a change in breeding targets was needed. The development of multiple cropping systems will also demand interactions between agronomists

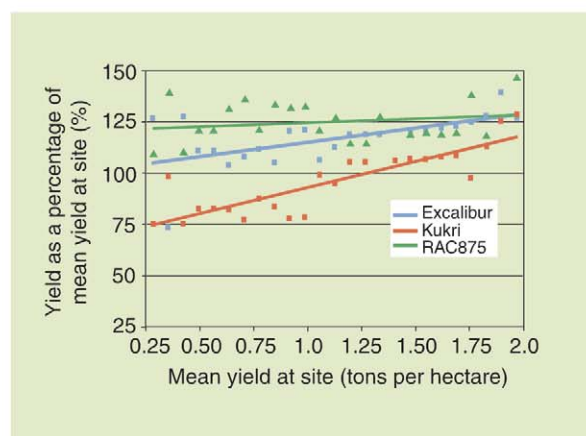


Fig. 2. Yield under severe drought stress. Shown are differences in maintenance of yield with lower water supply for three lines of Australian bread wheat. Low-yielding environments are water-limited fields in southern Australia. The yield for each of the three lines is plotted relative to the average yield for that site of at least 50 independent genotypes. The lines were evaluated in 25 environments (multiple sites for several years).

Box 1. New breeding technologies.

MAS uses a marker such as a specific phenotype, chromosomal banding, a particular DNA or RNA motif, or a chemical tag that associates with the desired trait. For example, a DNA marker closely linked to a disease resistance locus can be used to predict whether a plant is likely to be resistant to that disease.

- Gene pyramiding can usually only be accomplished by using MAS. For example, pyramiding is used to create durable disease resistances by selecting for two or more resistance genes against a pathogen. Multiple, partial, rust-resistance genes in wheat can be accumulated into elite varieties to provide strong and durable resistance. Single genes would give only weak resistance, and MAS offers the only effective method for accumulating multiple resistances (22).

- Marker-assisted recurrent selection (MARS) involves crossing in selected individuals at each cycle of crossing and selection. In this way, desirable alleles can be brought into the breeding scheme from many different sources. This technique has been applied to sunflower, soybean, and maize to bring desirable alleles at several target loci into single elite lines (27).

- Genome-wide or genomic selection also relies on MAS and is under evaluation for the feasibility of incorporating desirable alleles at many loci that have small genetic effect when used individually. In this approach, breeding values can be predicted for individual lines in a test population based on phenotyping and whole-genome marker screens. These values can then be applied to progeny in a breeding population based on marker data only, without the need for phenotypic evaluation. Modeling studies indicate that this method can lead to considerable increases in the rates of genetic gain by accelerating the breeding cycles (20). In the oil palm, for example, this approach could lead to the release of improved germplasm after only 6 years as compared with the current time of 19 years (28).

- Complex trait dissection uses high-throughput technologies to determine the phenotypic components of complex traits. For example, robotic greenhouse systems use nondestructive imaging to monitor growth rates, stem and leaf architecture, and root structure (for example, see www.lemnatec.com/). Similar systems can also be adapted for the detection of characteristics of chlorophyll fluorescence (which indicate aspects of plant responses to the environment) or fluorescent protein–labeled genotypes.

- The analysis of complex traits has recently been bolstered by developments in statistical and modeling methods for the analysis of phenotypic data obtained from field and controlled environment studies. For example, in assessing drought tolerance in wheat and sorghum, modeling can be used generate an “index of the climatic environment” to identify the stages of crop development where there is the strongest interaction between genotype and the environment and to identify aspects of the crop response that can be most readily enhanced by breeding and selection (29).

- Increasing genetic diversity requires an expansion of the germplasm base in breeding programs (22), but this is dependent on enhancing techniques for assessing the value of the program and using individual accessions from germplasm collections. Improvements in phenotyping and genotyping will help remove this limitation by facilitating the identification and characterization of key adaptive QTLs. For example, increased expression of a boron transporter in a barley landrace leads to high tolerance to soil boron in elite varieties when the high-expression allele is transferred. Screening for variation in expression levels for this gene in germplasm collections may identify new sources of tolerance (30).

- Introgression of novel alleles from landraces and wild relatives is often slow and tedious, but options are now being developed for accelerating introgression as we learn more about the recombinational behavior of plant genomes and develop new breeding methods.

- The wider deployment of GM approaches will be needed for the introduction of novel genes and alleles from diverse sources, and particularly for traits that are absent from plant genomes (for example, *Bacillus thuringiensis* toxin from soil bacteria) or where there is insufficient variation for practical utility (for example, vitamin A accumulation in rice endosperm).

- The constraints on regulatory and consumer acceptance of GM can be reduced by adopting alternative approaches for engineering plants. For example, consumer acceptance may be greater and regulatory approvals simpler for plants transformed with cis-genic vectors in which only host gene sequences are used in the transformation construct (www.cisgenics.com/). Similarly, the creation of marker-free plants, where only the DNA that has a biological effect remains in the plant, has been used to develop plants without antibiotic-resistance genes, which has caused much controversy (31).

- Heterosis (hybrid vigor) for inbreeding species (that is, species that usually self-pollinate, such as rice and wheat) can offer 20% to over 50% yield increases, and, for example, a 68% increase in yield has been achieved in foxtail millet (32). Strategies for using heterosis more widely to increase yields in inbreeding crops center on finding ways of reducing the cost and increasing the efficiency of producing hybrid seed. These include identifying new sources of male sterility for hybrid creation [such as thermosensitive genic male sterility in rice (33)] and using GM approaches to engineer sterility and restore fertility (such as the InVigor Canola from Bayer CropScience)]. Another possible mechanism for producing hybrid seed involves the use of apomixis, where plants produce seed without the need for fertilization. This allows hybrid vigor to be fixed. Creating apomictic crop plants may also be possible as we learn more about the genes controlling this process.

- Direct targeting of key heterotic loci may also be achievable as we learn more about the molecular basis of hybrid vigor (for example, in maize) (34).

Limitations

Of course, none of this will happen without suitably trained staff in plant breeding and molecular biology, so substantial increases in the education of plant breeders are essential. Most countries are struggling to maintain strong breeding capabilities. A vital adjunct is the free communication of resources and capabilities from technology developers to technology users. Resource and capacity building within breeding programs is essential to develop novel approaches, particularly in developing countries. Furthermore, developing countries critically need support for the development of crops, where there has been little interest from the developed world and, consequently, little investment. In many cases, these “orphan crops,” such as cassava and plantain, are of critical importance for food security.

For many of the new breeding technologies, access to equipment, reagents, and skilled personnel is critical. Whereas service providers deliver this support to breeding programs in some parts of the world, they are often too expensive for poorly resourced breeding programs, and the logistics of sending plant tissue samples for analysis in a timely fashion can be prohibitive. Some organizations are attempting to address this limitation by establishing support services for breeding programs in the developing world (www.generationcp.org/).

and breeders. However, it is clear that more is required than can be provided by traditional breeding approaches.

Emerging Technologies for Crop Breeding

The production and evaluation of genetically modified (GM) crops is an active area of research, but the access of growers to this technology in many countries is currently restricted primarily because of political and bioethical issues (Box 1). Nevertheless, GM technologies permit the generation of novel variation beyond that which is available in naturally occurring (or even deliberately mutated) populations. Classic applications of GM include the use of proteinaceous toxins to control insect pests and “golden rice,” which is biofortified with vitamin A (11). Crucial to the future deployment of GM crops are the discovery and characterization not only of genes but of promoters that provide accurate and stable spatial and temporal control of the expression of the genes (12). Development of cis-genic vectors and marker-free transgenic plants (Box 1) may help to ease some of the political concerns about GM technologies. Nevertheless, the widespread application of GM technologies will remain limited while regulatory demands impose high costs on releasing GM crops (Box 1). Although it is likely that most of the important contributions to crop improvement in the coming 5 to 10 years will continue to be from non-GM approaches, we consider that transgenic technologies will inevitably be deployed for most major crops in the future.

Methods of crop breeding have undergone major changes, and a range of technologies is improving the rate and success of crop improvement in some breeding programs, but these have yet to be widely adopted. Contributions are being made through new selection strategies that are informed by sophisticated genetics, the use of computers to track and manage field trials, and biometric methods for field-trial design and assessment of interactions between genotype, environment, and management (13).

Marker-assisted selection (MAS) techniques (Box 1) are free of the political issues that have plagued the application of GM technologies. MAS involves using variation at the DNA level to track and monitor specific regions of the genomes during crossing and selection (14). The greatest benefit of MAS occurs where the target traits are of low heritability, are recessive in nature, and involve difficult and costly phenotyping, and where pyramiding of genes is desired for results such as disease and pest resistance. In these cases, MAS is likely to be more reliable, more convenient, or cheaper than phenotype-based selection, and MAS currently provides the only viable method for gene pyramiding. Molecular markers are also important in analyzing the mode of inheritance of certain traits and assess-

ing genetic diversity. In cases where desirable traits are closely linked and in repulsion, markers can be critical in selecting rare recombination events.

In many cases, MAS provides an important alternative to phenotypic selection. However, the success of markers depends on their reliability in predicting phenotype. Many key stresses associated with rapid environment changes, notably drought and salinity tolerance, are complex and highly variable. For these types of traits, it is necessary to dissect tolerance into component contributory traits and to identify genetic regions encoding the traits, rather than overall plant tolerance (6, 15, 16). However, this genetic approach requires high-throughput phenotyping (phenomics) (17) (Box 1). Phenomics also allows screening of populations for particular traits and will facilitate the introgression of novel variation from wild germplasm. Phenomics will enable tighter definition of the properties of molecular markers, allowing introgression of appropriate combinations of tolerance traits into commercial varieties for particular target environments.

The combination of reliable phenotyping and MAS has been particularly important in transferring desirable alleles by simple backcrossing into elite germplasm. Although MAS has been used to track multiple independent loci (18), conventional breeding schemes become quite complex as the number of target loci expands. To overcome the problems of dealing with multiple loci, in particular, multiple loci of small genetic effect, two relatively new methods involving MAS can be deployed: marker-assisted recurrent selection (MARS) and genome-wide or genomic selection (GWS) (19, 20) (Box 1). MARS involves crossing selected individuals at each selection cycle so that desirable alleles at the target loci are introduced one at a time or through the merging of multiple crossing and selection streams. A problem with this approach is that it is most effective for genes or quantitative trait loci (QTLs) of major effect. In contrast, GWS does not require prior information on marker trait associations and can be used to select for multiple loci of small genetic effect. In this approach, populations are extensively genotyped to give full genome coverage and phenotyped. Subsequently, these data allow the prediction of phenotypic performance of an individual on the basis of whole-genome marker surveys.

These new breeding and selection strategies rely on the availability of cheap and reliable marker systems. A serious limitation in marker application for some species has been the paucity of useful markers. However, the new sequencing platforms have allowed large-scale discovery of single-nucleotide polymorphisms (SNPs) for species where few markers were previously available. The new marker systems combined with the new marker-based selection and screening strategies

provide a base for a revolution in crop breeding and genetics.

Expanding the Germplasm Base for Plant Breeding

The success of plant breeding over the past century has been associated with a narrowing of the available genetic diversity within elite germplasm, particularly for some species such as peanut and soybean. New sources of variation include landraces and wild relatives of crop species, and although exploiting wild relatives as a source of novel alleles is challenging, it has provided notable successes in crop improvement. A particularly important example of the introgression of genetic information from a relative was the use of the short arm of rye chromosome 1R in wheat. In the early 1990s, this wheat-rye translocation was used in 45% of 505 bread wheat cultivars in 17 countries (21). Increasingly easy gene discovery, improved enabling technologies for genetics and breeding, and a better understanding of the factors limiting practical exploitation of exotic germplasm promise to transform existing, and to accelerate the development of new, strategies for efficient and directed germplasm use (Box 1).

Most crop geneticists agree that enrichment of the cultivated gene pool will be necessary to meet the challenges that lie ahead. However, to fully capitalize on the extensive reservoir of favorable alleles within wild germplasm, many advances are still needed. These include increasing our understanding of the molecular basis for key traits, expanding the phenotyping and genotyping of germplasm collections, improving our molecular understanding of recombination in order to enhance rates of introgression of alien chromosome regions, and developing new breeding strategies that permit introgression of multiple traits (22). Recent progress has shown that each of these challenges is tractable and within reach if some of the basic problems limiting the application of new technologies can be tackled.

Limitations in Applying the New Technologies

Several issues are likely to limit the application of these new methods, particularly for breeding programs in the public sector (Box 1). Regulatory complexity and high costs have prevented the widespread delivery of GM technologies (Box 1). Over the coming decade or so, however, it seems inevitable that GM technologies will become much more widely used—it is probably a case of “when,” not “if.” A consequence emerging for crops that are now dominated by GM varieties (such as cotton, soybean, and maize) is that breeding programs are now based around GM varieties, and consequently, breeding programs in non-GM jurisdictions have limited access to current advances. The key limitations for traditional breeding include lack of resources, training, and capabilities for most of the world’s

crop improvement programs (23, 24) (Box 1). It is important, therefore, that we expand the scope of and access to new marker platforms to provide efficient, cost-effective screening services to the breeders. Communication and mechanisms for delivery of material to breeders must be developed. There is an urgent need to expand the capacity of breeding programs to adopt new strategies. The clearly documented high rate of return on such investments in the past should be kept in mind (25).

The concerns about food security and the likely impact of environmental change on food production have injected a new urgency into accelerating the rates of genetic gain in breeding programs. Further technological developments are essential, and a major challenge will be to also ensure that the technological advances already achieved are effectively deployed.

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PERSPECTIVE

Smart Investments in Sustainable Food Production: Revisiting Mixed Crop-Livestock Systems

M. Herrero,^{1*} P. K. Thornton,¹ A. M. Notenbaert,¹ S. Wood,² S. Msangi,² H. A. Freeman,³ D. Bossio,⁴ J. Dixon,⁵ M. Peters,⁶ J. van de Steeg,¹ J. Lynam,⁷ P. Parthasarathy Rao,⁸ S. Macmillan,¹ B. Gerard,⁹ J. McDermott,¹ C. Seré,¹ M. Rosegrant²

Farmers in mixed crop-livestock systems produce about half of the world's food. In small holdings around the world, livestock are reared mostly on grass, browse, and nonfood biomass from maize, millet, rice, and sorghum crops and in their turn supply manure and traction for future crops. Animals act as insurance against hard times, and supply farmers with a source of regular income from sales of milk, eggs, and other products. Thus, faced with population growth and climate change, small-holder farmers should be the first target for policies to intensify production by carefully managed inputs of fertilizer, water, and feed to minimize waste and environmental impact, supported by improved access to markets, new varieties, and technologies.

“Business as usual” investments in agriculture, although necessary (1, 2), are unlikely to deliver sustainable solutions as the world rapidly changes (3, 4). At the recent G8 summit in Italy, the leaders of the world's wealthiest countries promised to invest U.S.\$20 billion to improve global food security. Most of that money is likely to flow to the developing world, where over the next few decades agricultural systems, already facing a va-

riety of stresses, will be expected to accommodate a massive population surge. Even an investment of this magnitude could fail to generate food security if its deployment is not well planned and based on sound science.

The usual culprits, such as inefficient aid delivery, government corruption, and political unrest, are a barrier to progress but are not the most important problem. Rather, it involves a fundamental failure to appreciate the range of dif-

ferent agricultural systems that are expected to feed our planet in the coming decades and their policy needs. The diverse pressures that are acting on agricultural systems in various parts of the world include population increase, rising incomes and urbanization, a rapidly rising demand for animal products in many developing countries, and a fierce competition for land and water (3, 5, 6), all of which will have profound effects on food security (1). Croppers and livestock keepers the world over have steadily accumulated local experience and knowledge that will help them to adapt in the future, but the rapid rates of change seen in many agricultural systems in developing countries may simply outstrip their capacity. Yet, recent scientific assessments (1, 2, 7–10) and the technical and policy recommendations that flow from them have not fully captured the complex biological, social, and economic dynamics of the variety of chal-

¹International Livestock Research Institute (ILRI), Post Office Box 30709, Nairobi, Kenya. ²International Food Policy Research Institute (IFPRI), 2033 K Street NW, Washington, DC 20006, USA. ³International Finance Corporation, The World Bank Group, Washington, DC 20433, USA. ⁴International Water Management Institute (IWMI), Colombo, Sri Lanka. ⁵Australian Centre for International Agricultural Research, Canberra, ACT, Australia. ⁶Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia. ⁷Independent consultant, Nairobi, Kenya. ⁸International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India. ⁹CGIAR System-wide Livestock Programme, Addis Ababa, Ethiopia.

*To whom correspondence should be addressed. E-mail: m.herrero@cgiar.org

challenges likely to confront future crop and livestock production (5).

Recently, the Consultative Group on International Agriculture Research (CGIAR) considered the issues facing mixed crop and livestock production, one of the predominant forms of agriculture in the developing world (3). Mixed systems enable the farmer to integrate different enterprises on the farm; in such systems, livestock provide draft power to cultivate the land and manure to fertilize the soil, and crop residues feed livestock (Fig. 1). Moreover, income from livestock may be able to buffer low crop yields in dry years. These mixed systems may be used intensively close to urban markets, as well as in less productive areas with limited market access.

The synergies between cropping and livestock husbandry offer many opportunities for the sustainably increasing production (11) by raising productivity and increasing resource use efficiency both for households and regions. This, in turn, can increase incomes and secure availability and access to food for people while maintaining environmental services. However, during the next 20 years mixed crop-livestock farmers may not be able to stay abreast of population growth, environmental change, and the increasing demand for animal products (1, 3).

According to the CGIAR analysis, the world's one billion poor people (those living on less than \$1 a day) are fed primarily by hundreds of millions of small-holder farmers (most with less than 2 ha of land, several crops, and perhaps a cow or two) and herders (most with fewer than five large animals) in Africa and Asia (3). Furthermore, mixed crop-livestock systems could be the key to future food security; two-thirds of the global population already live in these sys-

Box 1. Enhancing livestock productivity through improved dual-purpose crops.

In developing countries, some crops like maize, wheat, sorghum, and millet are dual purpose: Their grain provides food for humans and their residues are used as feed for livestock. Traditionally these crops have been bred to improve grain yield and drought and pest resistance. However, in the past decade it has been recognized that farmers in mixed crop-livestock systems value the crop residues sometimes as much as the grain owing to their importance as a feed for livestock, particularly in the dry season (29). Breeding programs for these crops are increasingly being adapted to include breeding for residue quality without compromising the original objectives associated with increasing grain yield.

In India, where the demand for crop residues as feed is very high, improved dual-purpose varieties of sorghum and millet have had significant impacts on the productivity and efficiency of crop-dairy systems. Small-holders have been able to increase the milk production of buffalos and cows by up to 50% while at the same time obtaining the same grain output from their crops. This has increased the demand for dual-purpose crops with relatively high-quality crop residues, and burgeoning fodder markets have developed around cities like Hyderabad (29).

tems, and much of the future population growth will occur there. Already, mixed systems produce close to 50% of the world's cereals and most of the staples consumed by poor people: 41% of maize, 86% of rice, 66% of sorghum, and 74% of millet production (3). They also generate the bulk of livestock products in the developing world, that is, 75% of the milk and 60% of the meat, and employ many millions of people in farms, formal and informal markets, processing plants, and other parts of long value chains (3).

Intensive Crop-Livestock Systems

The pressures currently acting on the so-called "high-potential," intensively farmed lands of developing countries are large enough to slow and possibly end the substantial increases in growth rates of crop production seen during recent decades. For example, diminishing water resources are becoming a huge constraint to rice and wheat

production in South Asia (1). There, livestock numbers are projected to increase significantly: cattle and buffalo from 150 to 200 million animals by 2030 and pigs and poultry by 40% or more in the same period (1, 3). Pressures on biomass to feed these animals are already high, with trade-offs in the use of resources (land, water, and nutrients) becoming increasingly hard to balance in these systems, especially as competition for biomass for food, feed, fertilizer, and fuel increases (3, 12, 13). Similar caps on natural resources in the East African highlands and other high-potential agricultural areas of Africa are appearing in the form of infertile soils, degraded lands (13, 14), depleted water sources, carbon losses, shrinking farm sizes, and decreasing farm productivity (14, 15). Recent research suggests that some of these areas may not respond to increased fertilizer inputs and will need a closer integration of livestock and crop production to improve productivity (14, 15).

The key will be to develop sustainable intensification methods that improve efficiency gains to produce more food without using more land, water, and other inputs (3, 16, 17). For example, in parts of Asia there is considerable scope to produce more meat and milk in mixed systems through more efficient production systems (Box 1). Over the past 30 years, researchers have doubled the efficiency with which chickens and pigs convert grain into meat (6, 16), and this has resulted in less grain consumption per unit of poultry and pig meat produced. Although global poultry and pork prices have decreased significantly, this has been at the expense of increasing the price of cereals available for human consumption (1) and has promoted deforestation in the neotropics (16, 18).

In some regions, farmers will have to change the species of livestock they keep to use their resources more efficiently, and policies to promote livestock specialization will be needed. A measurable shift is already taking place in South Asia's intensive mixed crop-livestock systems,

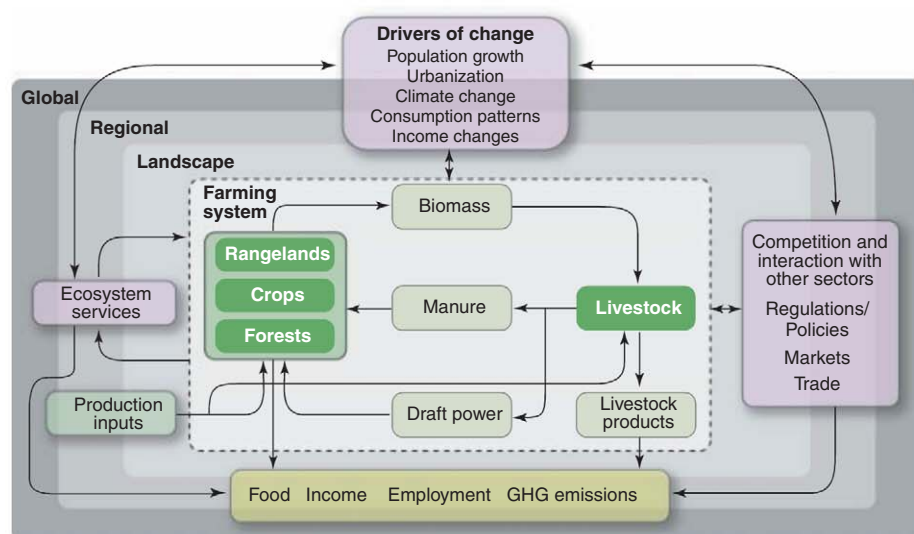


Fig. 1. Main interactions in mixed crop-livestock systems in the developing world.

from ruminant and crop production to intensive industrial poultry. Here, rates of growth in poultry production are projected to exceed 7% per year by 2030, which is two- to threefold higher than rates of growth for ruminants or crop production (3). Specialization and intensive industrial livestock production will in turn require environmental and trade regulations. For example, in parts of Asia, large numbers of pigs in unregulated intensive industrial systems pollute water sources in peri-urban areas (19) (Fig. 2). Concentration of animals can also increase the risk of outbreaks of emerging infectious diseases, afflicting livestock and people alike (20) (Box 2).

Extensive Crop-Livestock Systems

Significant contributions to future food security could be made in the more extensive mixed crop-livestock systems used in developing countries, where there is less pressure on the land and the crop productivity is far from optimal (21). For example, yields of dryland crops such as sorghum, millet, groundnut, and cowpea could easily be increased by a factor of three with appropriate land preparation, timing of planting, and use of fertilizers and pesticides (21). In specific circumstances, genetically modified (GM) crops can be an important contribution to improving crop productivity by increasing water use efficiency or reducing the impacts of pests and diseases. Policies and public investments in infrastructure and market development will be essential to create systems of incentives, reduce transaction costs, and improve risk management (10, 22). Integration of production in these systems to supply agro-ecosystems services to the more-intensive systems will also be needed to ensure sustainability (3).

Investing in extensive mixed systems will require considerable changes in public investments. Instead of allocating most resources to highly populated areas or those with high agricultural potential, developing-country governments will have to begin investing in infrastructure and services for more extensive areas (22), many of which are likely to be affected by climate change in the future (2). With better roads, markets, health facilities, and other infrastructure and services, the rural-to-urban migration rates in the extensive mixed areas could be reduced (10), thus nurturing the next generation of food producers.

Conserving Agro-Ecosystems

In developing regulatory frameworks for sustainable food production, we need to define the limits to agricultural intensification (11, 16). Lessons can be learned from the developed

Box 2. Intensification and the risks of avian influenza.

Recent outbreaks of avian influenza in both domestic poultry and the human population have been a source of considerable concern. The disease, caused by the highly pathogenic H5N1 virus, appears to move between poultry and wild birds and to people. The virus was identified in domesticated geese in southern China in 1996 and in humans in Hong Kong in 1997. H5N1 avian influenza then spread rapidly in 2002, with outbreaks in poultry, wild birds, and other mammalian species in more than 60 countries. By the end of 2009, 467 human cases and 282 deaths had been reported to the World Health Organization (30). In response, more than 200 million poultry have been killed by the virus or culled to prevent its spread.

The epidemiology of the disease is not well understood because there are many vectors, including wild birds and other wildlife. However, large concentrations of birds in both backyard and intensive systems, coupled with poor disease control or underfunded veterinary services in some developing countries, could be significant risks for the spread of the disease. The risks of livestock diseases, including those associated with intensifying systems, will need to be addressed through developments in disease surveillance and early warning systems.

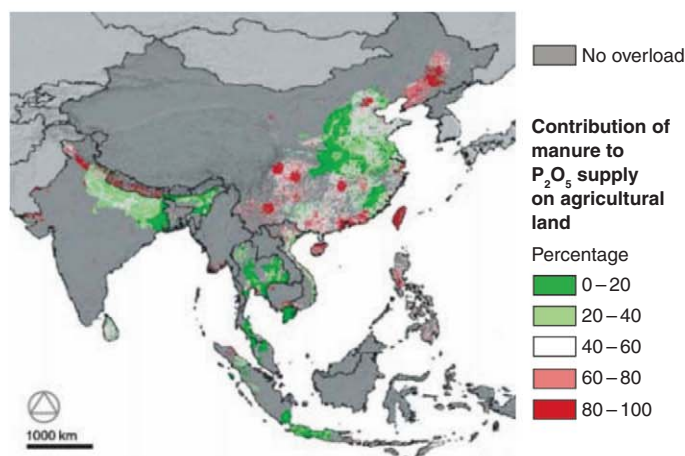


Fig. 2. Contribution of livestock to estimated nutrient overloads in Asia (19).

world in terms of matching efficiency gains and environmental regulation. For example, the European livestock sector grew slightly in the past decade while reducing greenhouse gas emissions by 9% (23). Particularly in the developing world, we need to determine criteria for defining intensification thresholds at local levels before irreversible environmental degradation occurs (16).

Any agricultural investment portfolio funded by the G8 should be sufficiently diverse to include payments for protecting water, carbon, biodiversity, and other global goods and ecosystem services where rangeland and other systems are under significant pressures and need to de-intensify or stop growing altogether (3, 24, 25). For example, implementing schemes to pay farmers for protecting water towers in the Himalayas could be key for food production in large parts of South Asia. Such schemes could be aimed at sustaining stream flow early in the growing season, when water inputs are critical for crop production (26).

Relatively modest extra investments could halve child malnourishment rates in developing

countries (currently 27%), in spite of projected population growth to 2050 (27).

Nevertheless, to reduce poverty while increasing food supplies and maintaining functional ecosystems will require well-regulated and differential growth in crop and livestock production (1, 3, 6). It will require public and private investments in the more-extensive mixed agricultural systems neglected in the past (22). It will require higher public and donor funding for research and development in the livestock sector, which historically has been lower than those for food crops, often by a factor of 10 or more (28). It will require

differentiated and nuanced policies able to assess the trade-offs between agro-ecosystem services and human well-being (5). And it will require that governments and donors, together with scientists and other stakeholders, precisely target technological, investment, and policy options to suit different farming systems and regions (3).

There is no doubt that agriculture as an engine for growth is regaining recognition by governments in developing countries (10). Together with the commendable and significant financial commitments of G8 countries to developing-country agriculture, they now need to match it with an intellectual commitment—one that embraces a new agricultural frontier and new efficiencies, incentives, and regulations in the food systems of developing countries.

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PERSPECTIVE

Measuring Food Insecurity

Christopher B. Barrett*

Food security is a growing concern worldwide. More than 1 billion people are estimated to lack sufficient dietary energy availability, and at least twice that number suffer micronutrient deficiencies. Because indicators inform action, much current research focuses on improving food insecurity measurement. Yet estimated prevalence rates and patterns remain tenuous because measuring food security, an elusive concept, remains difficult.

The 2008 global food price crisis, which sparked riots in more than two dozen countries, rekindled political and scientific interest in food security. In their July 2009 joint statement, the G8 heads of state agreed "to act with the scale and urgency needed to achieve sustainable global food security" (1). To direct scarce resources to where they can do the greatest good, actions must be guided by reliable information as to who is food insecure, where, when, and why. This requires improved measurement of food insecurity and its causes and greater attention to key institutional and policy lessons learned.

An Elusive Concept

Among the various definitions currently in use, the prevailing definition, agreed upon at the 1996 World Food Summit, holds that food security represents "a situation that exists when all people, at all times, have physical, social and

economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life." This high standard encompasses more than just current nutritional status, capturing as well vulnerability to future disruptions in access to adequate and appropriate food (2, 3).

Food security is commonly conceptualized as resting on three pillars: availability, access, and utilization. These concepts are inherently hierarchical, with availability necessary but not sufficient to ensure access, which is, in turn, necessary but not sufficient for effective utilization (4). For most of human history, lives were short and unhealthy due in large measure to insufficient macronutrient (carbohydrate, fat, and protein) intake. Beginning in the 18th century, however, a succession of countries broke free of the nutritional poverty trap (5, 6), thanks largely to increased food availability made possible by advances in agricultural production; hence, the common association of food security with supply-side indicators, typically measured in daily calories per person.

Adequate availability is necessary, but does not ensure universal access to "sufficient, safe

and nutritious food." Access is most closely related to social science concepts of individual or household well-being: What is the range of food choices open to the person(s), given their income, prevailing prices, and formal or informal safety net arrangements through which they can access food? As Nobel Laureate Amartya Sen wrote, "starvation is the characteristic of some people not *having* enough food to eat. It is not the characteristic of there *being* not enough food to eat. While the latter can be a cause of the former, it is but one of many *possible* causes" (7). Access reflects the demand side of food security, as manifest in uneven inter- and intrahousehold food distribution and in the sociocultural limits on what foods are consistent with prevailing tastes and values within a community. Access also accentuates problems in responding to adverse shocks such as unemployment spells, price spikes, or the loss of livelihood-producing assets. Through the access lens, food security's close relationship to poverty and to social, economic, and political disenfranchisement comes into clearer focus. But because access is an inherently multidimensional concept, measurement becomes more difficult than with availability (4).

Utilization reflects concerns about whether individuals and households make good use of the food to which they have access. Do they consume nutritionally essential foods they can afford, or do they choose a nutritionally inferior diet? Are the foods safe and properly prepared, under sanitary conditions, so as to deliver their full nutritional value? Is their health such that they absorb and metabolize essential nutrients? Utilization concerns foster greater attention to dietary quality, especially micronutrient deficiencies associated with inadequate intake of essential minerals and vitamins.

Department of Applied Economics and Management, Warren Hall, Cornell University, Ithaca, NY 14853-7801, USA.

*To whom correspondence should be addressed. E-mail: ccb2@cornell.edu

In practice, analysts use proxy measures for different aspects of food security. Choice among indicators necessarily involves trade-offs, so the objective necessitating measurement commonly drives the choice of indicator.

Measures based on higher-cost individual and household surveys can associate measures with targetable individual characteristics, offering depth in measuring two or three of the pillars. Examples include the coping strategies index (8) and food expenditure and dietary diversity (9) measures, which rely on household or individual responses to survey questions about approaches to respond to shocks and past consumption, respectively. Likewise, hunger refers to the physical discomfort caused by a lack of food and can only be properly gathered at the individual level. Underweight summarizes individual anthropometric measures—such as weight-for-height, weight-for-age, or mid upper-arm circumference—at least two standard deviations below global reference values. Undernutrition reflects insufficient dietary energy (caloric) intake, according to internationally agreed standards (10). Malnutrition refers to undernutrition, obesity, and micronutrient (mineral and vitamin) deficiencies.

Simpler food availability measures enable frequent and geographically broad estimates, but at the expense of neglecting waste and the inevitably unequal distribution and uses of food within a population. The low cost and relative ease of generating availability estimates, especially at a national and global scale, account for the lasting appeal of such measures long after the food security community fully absorbed Sen's message.

Measurement matters for at least three major reasons. First, each measure captures and neglects different phenomena intrinsic to the concept of food security, thereby subtly influencing prioritization among food security interventions. Historically, reliance on national food availability estimates has focused attention on food aid shipments and agricultural production strategies to increase food supplies in the short and long term, respectively. Over roughly the past quarter century, Sen's core thesis—that food access accounts for most food insecurity—has focused increased attention on individual-specific hunger and underweight data, which naturally reinforces strategies based on poverty reduction, food price, and social protection policies. For example, the voluntary guidelines on the right to ade-

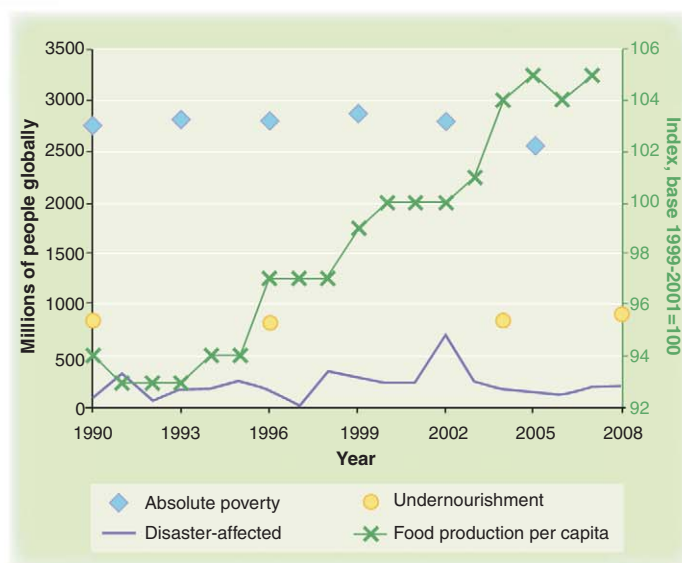


Fig. 1. Different food security proxy indicators paint different pictures. Undernourishment is far greater than just those affected by disasters but much less than those living in absolute poverty. And none of those measures show the improvement over time that food availability measures do. Data sources: Absolute poverty (<\$2/person per day): (31); Undernourishment: (32); Disaster-affected populations: (33). Gross food production per capita (base 1999–2001 = 100) (34). Food production series to be read against right-hand vertical axis; all other series against left-hand vertical axis. Unfortunately, no comparable series are available for micronutrient malnutrition or for perceptions-based measures.

quate food, unanimously agreed by all U.N. Food and Agriculture Organization (FAO) member states in 2004 (11), was a response to lack of progress in individual-level indicators despite growth in aggregate food supplies and incomes.

Second, observational data necessarily report on the past. But policy-makers are most interested in the likely future effects of prospective interventions. An ideal food security indicator would reflect the forward-looking time series of probabilities of satisfying the access criteria (3). Yet there has been little effort to date in testing the forecasting accuracy of currently available indicators (12).

Third, national-level measures inherently lend themselves only to addressing national-scale food availability shortfalls, not intranational access and utilization concerns. Insofar as food insecurity measures diagnostically inform actions, they must be readily associated with targetable characteristics of vulnerable households and individuals and remediable causal factors that lead to food insecurity. The research frontier therefore revolves around the development of cross-nationally comparable, longitudinal monitoring and analysis at the household and individual level.

Patterns and Trends

The most widely cited food insecurity figures are the “undernourishment” estimates generated by

FAO, derived from national-level food balance sheets and strong assumptions about intranational distribution of food. Alternative measures, such as those reported to Congress each year by the U.S. Department of Agriculture (USDA), generated by simulation models based on prices and national accounts and production equations, often differ radically from FAO estimates. For example, in June 2009, FAO estimated that the number of undernourished people globally climbed to 1020 million (13) (Fig. 1); in the same month USDA estimated only 833 million food insecure (14). And the FAO estimate for Asia was more than two-thirds higher than USDA's (642 versus 379 million) while that for Sub-Saharan Africa was nearly one-third lower (265 versus 385 million). Such discrepancies make even macroscale geographic targeting difficult for policy-makers.

The global figures mask considerable heterogeneity among and within regions, especially in trends. In China and Southeast Asia, tens of millions fewer people suffer undernutrition than a generation ago due to broad-based, rapid economic growth. In other regions, including South Asia and parts of east and southern Africa, undernutrition rates have fallen even while the number of undernourished has increased due to population growth. And in some regions (e.g., central Africa), both numbers and rates have increased (15).

Continued reliance on contested national food availability measures reflects the limited availability and timeliness of household and individual data collected in nationally representative surveys. A growing literature proves the value of survey data that capture objective dietary, economic, and health indicators as well as subjective measures of adequacy, risk exposure, and sociocultural acceptability (16–18). Food security measures based on household and individual data routinely generate higher estimates of food insecurity than those derived from more aggregate data. The differences seem attributable to differences not only in intra- and interhousehold nutrient distribution but also in the resulting estimates of nutrient availability (17). Not surprisingly, survey-based estimates of food insecurity are more strongly correlated with poverty estimates, which are likewise generated from household survey data.

Beyond the increased precision that more disaggregated evidence allows, individual- and household-level survey-based measures permit reasonably accurate prediction of who is most

likely to be affected adversely by potentially harmful shocks, such as food price increases, drought, or slumping demand for wage labor. Survey data-based predictions of community-level variables, such as child undernutrition, can even underpin catastrophe insurance contracts that trigger payouts when most needed (19). By contrast, aggregate food availability is a poor predictor of other food insecurity indicators. For example, the undernourished population has increased by 9% globally despite a 12% rise in global food production per capita since 1990 (Fig. 1).

Although the most severe food insecurity is typically associated with disasters such as drought, floods, war, or earthquakes, most food insecurity is associated not with catastrophes, but rather with chronic poverty (Fig. 1). Only 8% of hunger-related deaths worldwide in 2004 were caused by humanitarian emergencies; 92% were associated with chronic or recurring hunger and malnutrition (20). Similarly, in every country, rates of child stunting—reflecting chronic undernutrition—far exceed those of child wasting—indicating short-term, acute undernutrition—with the difference greatest in the poorest countries.

Because most food insecurity is seasonal or regular but aperiodic—i.e., associated with temporary unemployment, episodes of ill health, or other recurring adverse events—people anticipate such possibilities and routinely engage in precautionary behaviors to try to mitigate their risk. Hence perceptions-based survey measures consistently find food insecurity rates several times higher than related hunger or insufficient-intake measures (21).

Even perceptions data may not suffice to capture utilization problems, such as those associated with micronutrient malnutrition. The prevalence of micronutrient deficiencies is imprecisely known; rough estimates suggest that iodine, iron, vitamin A, and zinc shortfalls alone affect at least 2 billion people, disproportionately women and children. This leads to increased risk of both chronic and infectious disease, aggravates diseases' effects, and leads to irreversible loss of cognitive and physical function, especially during the crucial period from –9 to 24 months of age, during which children are biologically vulnerable and completely dependent on caregiver knowledge to utilize foods appropriately (22–25). These irreversible effects foster persistent poverty, reinforcing the consequences of food insecurity.

Policies and Institutions for Effective Intervention

Effective, direct food security interventions depend on effective targeting of the vulnerable subpopulation(s) and of the causes of insecurity, as well as prompt response. Where data collection is timely, causal factors can be robustly associated with food insecurity measures,

and when predictive models have demonstrable accuracy, preventive measures can substantially reduce unnecessary human suffering. The long-term consequences of crises can be limited where appropriate policies and institutions are already in place, such as social protection schemes to cushion people in times of adversity and early childhood health programs to protect the most vulnerable from even short-lived interruptions in essential nutrient intake.

Automatic stabilizer and safety net programs are important means of circumventing inconsistent or inadequate government and donor response. Political and economic elites are rarely severely affected by crises, nor do they suffer chronic food insecurity. Because they rarely face intense, immediate political pressure, slow, halting or incompetent government and donor response is a recurring problem. For example, the median delivery time for emergency food aid from the United States, the main global food donor, is nearly 5 months due to legislative restrictions on procurement and shipping (26). And even prescient early-warning systems often go unheeded. In the Niger crisis of 2004–2005, for example, below-normal rains and anticipated locust attacks led to a low cereals harvest that elicited prompt government and United Nations appeals for emergency assistance in November 2004. But the global response was anemic. By July 2005, the Niger situation was finally attracting graphic global media coverage that led to a significant global response, much of which arrived with the next harvest.

These delays are both deadly and expensive. In Niger, quite apart from the still unclear human health toll and lives lost to delays, the cost per beneficiary for World Food Programme deliveries more than tripled from February to August 2005, from \$7 to \$23, due to far greater need for supplemental and therapeutic foods instead of cheaper, bulk commodities, and the need for airlift and other quicker, but more expensive, logistical support (19). Poorly conceptualized or implemented relief programs can adversely affect communities, leaving them more vulnerable to food insecurity by displacing commercial food trade, affecting local prices, or distorting incentives and behaviors; hence, the need for prearranged financing facilities, social protection programs such as employment guarantee schemes, and other ready-made responses to emergent food security crises.

Perhaps the most important factor determining the efficacy of food security interventions is the quality of targeting. Does assistance reach the intended beneficiaries? Good targeting is exceedingly difficult. The neediest individuals are not always easily identified, even in participatory or community-based targeting efforts, for example, due to social isolation or discrimination. Effective targeting is usually based on a mixture of geographic indicators, observable

individual or household characteristics, program restrictions (such as work requirements) that induce self-selection out of participation by the non-needy, or community consultations. Identifying the most needy and the optimal form of assistance involves trade-offs across time, efficacy, and cost and commonly requires triangulation using multiple indicators across time and levels of aggregation (3, 26). In responding to transitory food insecurity associated with sudden natural disasters (e.g., earthquakes, hurricanes), careful and expensive data collection may be inappropriate, especially for short-lived interventions (27). By contrast, careful targeting is essential for long-term programs that address more common chronic food insecurity.

The greatest food security gains typically come not directly, from feeding programs, but rather indirectly, through policies that promote poverty reduction through employment creation and productivity growth among the poor, as well as safety nets to safeguard the vulnerable nonpoor. Enhanced control over productive assets and access to the technologies and markets necessary to sustainably use them to generate a stable livelihood are especially crucial to reducing vulnerability to food insecurity and facilitating the escape from poverty traps (28); hence, the importance of continued efforts to boost crop productivity, especially for micronutrient-rich foods, where food availability remains limiting, as is true of dozens of low-income countries.

Conclusions

Measurement drives diagnosis and response. As global attention returns to food security, new opportunities emerge to improve its measurement. Research is appropriately and increasingly moving toward survey-based anthropometric and perceptions measures to improve the disaggregated identification of food-insecure subpopulations and their targetable characteristics and behaviors. But the greatest advances in the measurement of food insecurity will come from three developments. First, a global network of sentinel sites using a standardized core survey protocol for regular, repeated household- and individual-level monitoring would enable us to track the coevolution of multiple food security indicators with targetable individual, household, and community characteristics across continents and to rigorously monitor and evaluate the impacts of various policy and project interventions. The recent review of social sciences within the CGIAR calls for this, modeled in part on the National Science Foundation's Long-Term Ecological Research network (29). Second, if we knew better the predictive accuracy of different indicators in forecasting future food security states, we could more cost-effectively concentrate data collection on measures of which targetable actions can be most reliably programmed.

This would help overcome the breadth-versus-depth trade-offs that presently limit the availability of suitable time series data at the individual and household level. Finally, just as poverty research is moving beyond static, snapshot measures to dynamic mobility ones, especially with respect to critical behavioral thresholds (28), so must the food security research community begin developing measures based on longitudinal data that capture the risk of food insecurity that respondents routinely voice in perceptions-based measures.

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PERSPECTIVE

Precision Agriculture and Food Security

Robin Gebbers^{1*} and Viacheslav I. Adamchuk²

Precision agriculture comprises a set of technologies that combines sensors, information systems, enhanced machinery, and informed management to optimize production by accounting for variability and uncertainties within agricultural systems. Adapting production inputs site-specifically within a field and individually for each animal allows better use of resources to maintain the quality of the environment while improving the sustainability of the food supply. Precision agriculture provides a means to monitor the food production chain and manage both the quantity and quality of agricultural produce.

To secure food supplies for the future requires adequate quantities and quality of agricultural produce, intensive yet envi-

ronmentally safe production, and the sustainability of the resources involved. In addition, the ability to track food materials from production through processing, storage, and retail provides added capability to respond to changing market conditions, ensure proper food nutrition and safety, and affect national and international policies related to food security.

Precision agriculture, or information-based management of agricultural production systems,

emerged in the mid-1980s as a way to apply the right treatment in the right place at the right time (1–3). Increasing awareness of variation in soil and crop conditions, combined with the advent of technologies such as global navigation satellite systems (GNSSs), geographic information systems (GISs), and microcomputers, serve as the main drivers (1, 2). Initially, precision agriculture was used to adapt fertilizer distribution to varying soil conditions across an agricultural field. Since then, additional practices have evolved, such as automatic guidance of agricultural vehicles and implements, autonomous machinery and processes, product traceability, on-farm research, and software for the overall management of agricultural production systems.

Apart from field crop production, precision agriculture technologies have been applied successfully in viticulture and horticulture, including orchards, and in livestock production, as well as pasture and turf management. Applications range from the tea industry in Tanzania and Sri Lanka to the production of sugar cane in Brazil; rice in China, India, and Japan; and cereals and sugar beets in Argentina, Australia, Europe, and the United States (4). Despite differences in the types of technology and the areas of adoption, the goals of precision agriculture are threefold. First, to optimize the use of available resources to increase the profitability and

¹Department of Agricultural Engineering, Leibniz-Institute for Agricultural Engineering (ATB), Max-Eyth-Allee 100, D-14469 Potsdam, Germany. ²Biological Systems Engineering Department, University of Nebraska-Lincoln, 203 Chase Hall, Lincoln, NE 68583-0726, USA.

*To whom correspondence should be addressed. E-mail: rgebbers@atb-potsdam.de

sustainability of agricultural operations. Second, to reduce negative environmental impact. Third, to improve the quality of the work environment and the social aspects of farming, ranching, and relevant professions (3). Because of the diversity of applications and scenarios, it is difficult to quantify the benefits of precision agriculture in general. In a review of 234 studies published from 1988 to 2005 (5), precision agriculture was found to be profitable in an average of 68% of the cases.

Variation in Soils and Crops

Important characteristics of the crop production environment, such as water and nutrient supply, often vary considerably over space and time within a single agricultural field (Fig. 1). Spatial variation in crop performance can be caused by soil as well as by diseases, weeds, pests, and previous land management. Variability over time arises from weather patterns and management practices. In particular, lack of nutrients, water stress, or plant diseases may form spatial patterns that change from year to year.

Relevant properties of soil productivity are soil moisture, clay content, organic matter content, nutrient availability, pH, and bulk density. Traditionally, these properties have been measured by soil sampling and offsite laboratory analysis or by on-the-spot measurement (such as measuring cone penetrometer resistance). Seasonally varying crop growth conditions

such as water stress, lack of nutrients, diseases, weeds, and insects have been evaluated by visual inspection and laboratory analysis of plant tissue. The relatively coarse sampling/measurement density of these conventional strategies may not be sufficient to reveal variation (6).

Soil and Crop Sensing

Remote and proximal sensing technologies have been introduced to improve spatial resolution. Remote sensing relies on acquiring images via optical and radiometric sensors installed on an aerial platform or a satellite, whereas proximal sensing systems are ground-based (mounted on a vehicle or carried by hand) and linked to a GNSS receiver. The advantage of remote sensing is that images of the entire field can be captured in one shot, whereas proximal soil sensors have to be moved across the landscape to create high-density measurements that can be mapped.

There is an enormous diversity of remote sensing data (7). The ground resolution, number and width of spectral bands, and timing of data collection differ among different service providers. Although remote sensing is useful for evaluating crop conditions, it provides a poor representation of the root zone environment, because the data represent the reflectance of the surface material, which might be bare topsoil, plant material, or a mixture of both.

Proximal soil sensing allows for a more direct detection of soil attributes than remote sensing (8). Three types of sensors are commercially available: electrical or electromagnetic sensors that measure electrical resistivity/conductivity or capacitance; optical sensors that obtain visible and near-infrared (Vis-NIR) spectra from within the soil; and electrochemical sensors that use ion-selective membranes to detect the activity of ions such as hydrogen, potassium, or nitrate. Soil compaction sensors for site-specific tillage will also be available in the near future.

Ultimately, the crop supplies the best indicator of variable growing conditions, and yield maps are most frequently used to evaluate crop performance. Yield maps summarize the overall impact of natural conditions, such as weather and soils, and of management activities. The observed spatial variation in quantity and quality of the harvest obtained by yield maps is directly related to the locally defined profitability.

In intensive crop production, the input of water, nitrogen, and agrochemicals for plant protection is usually regulated during the growing season. Vis-NIR reflectance spectroscopy is used to estimate plant biomass, chlorophyll content, and/or nitrate stress (9). Detection and identification of weeds via machine vision systems is also feasible, whereas other crop-status sensing techniques, such as laser fluorescence, thermal imaging, and ultrasonic proximity sensing, are still in the research stage (10). A recent application of imaging spectroscopy has been used to detect fungal infection in wheat, which, by allowing selective harvesting, may help to reduce the concentration of harmful mycotoxins (11).

Decision-Making

A typical cropping cycle that involves precision agriculture is shown in Fig. 2. Differentiated treatment of an agricultural field can be pursued using either a predictive or a reactive approach. In the predictive approach, information from yield history, thematic soil maps, field topography, and other spatial data records is used to predict variable crop performance and input needs. If a particular soil treatment can eliminate a yield-limiting factor that occurs in specific areas of the field (such as low soil pH or compaction), variable-rate technology can be used to solve the problem, at least temporarily. If the yield-limiting factor is expensive or impossible to remove (such as poor water-holding capacity in a non-irrigated field), it makes sense to reduce the quantity of inputs applied because they will never be consumed by the crop and will most likely be wasted in the environment.

In the reactive approach, rates of agricultural chemicals are varied according to the crop status at a given place and time. This requires real-time sensing and online application. It is

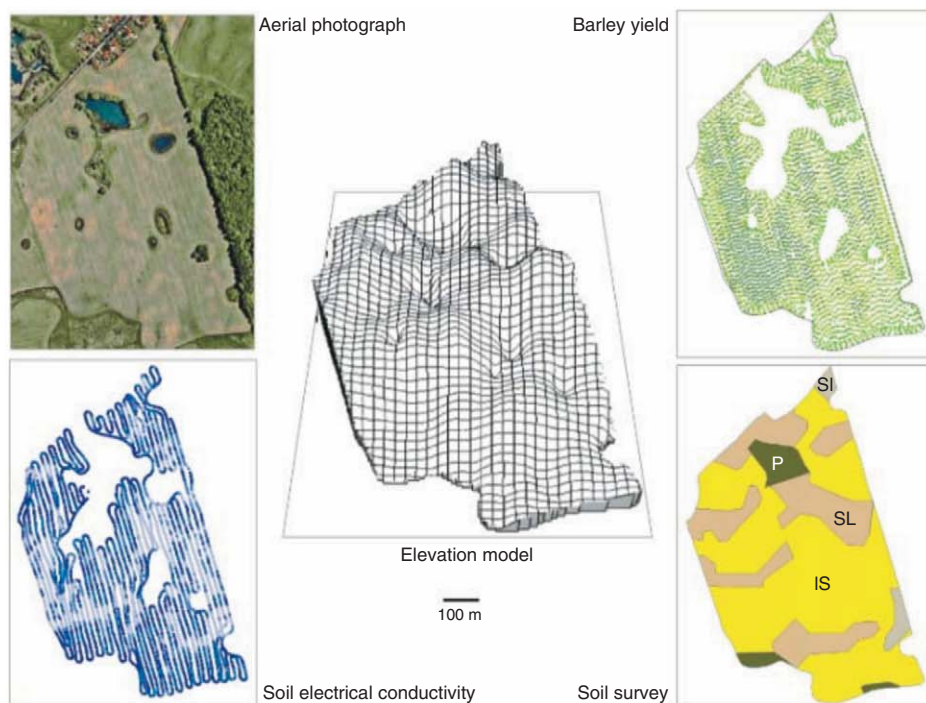


Fig. 1. Within-field variability in a ground moraine landscape (Wilmsdorf, Germany, 13°49'E, 53°09'N). The legend for the soil survey is as follows: P, peat soils; IS, slightly loamy sand; SL, loamy sand; SL, sandy loam.

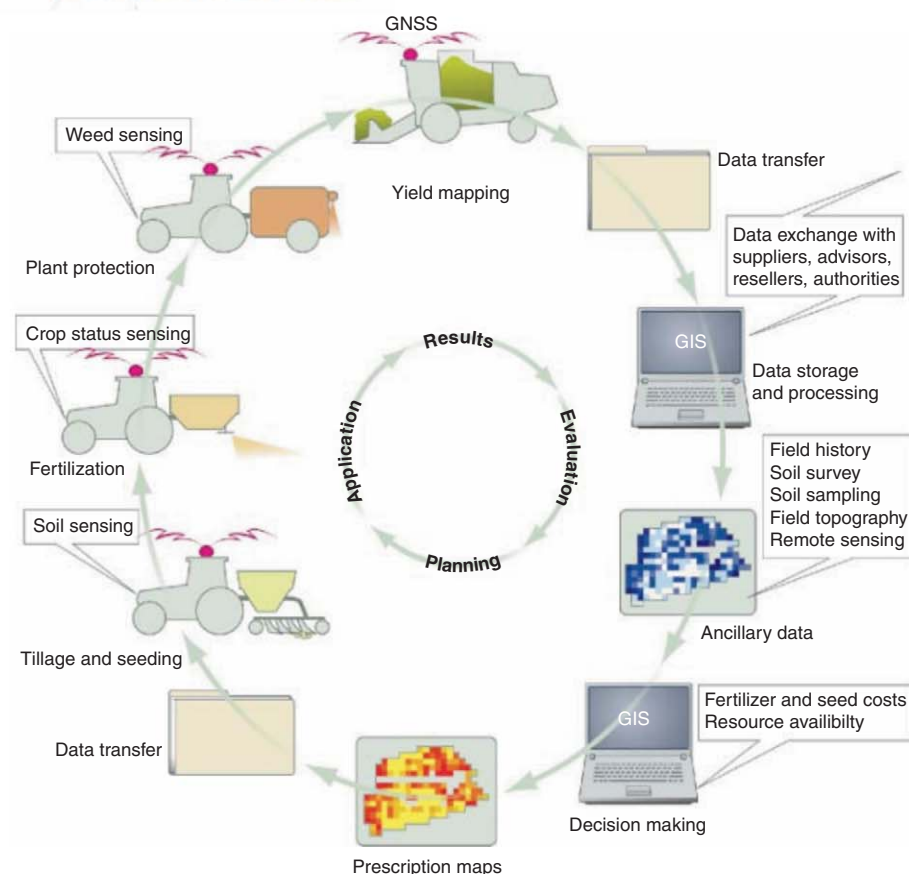


Fig. 2. Precision agriculture information flow in crop production [after (19), modified].

often used for the application of nitrogen fertilizers, for plant protection agrochemicals, and for water management. For instance, a relatively low chlorophyll content, which can be detected by real-time analysis of crop canopy reflectance in the Vis-NIR spectrum, may indicate the need for additional nitrogen or irrigation (9).

Precise Application, Guidance, and Automation

Implements for site-specific management are available for most tasks, including tillage, sowing, mechanical weeding, and the distribution of fertilizers and other agrochemicals (Fig. 2). To date, GNSS-based vehicle guidance has been the most widely used precision agriculture technology (12). It allows the operation of agricultural vehicles along parallel tracks or on predefined paths, which results in less stressful driving, along with significantly fewer gaps and overlaps. Originally, navigation aids were used to assist operators to steer agricultural vehicles using visual feedback such as light bars or graphical displays. Recent auto-guidance systems steer agricultural vehicles without direct input from operators. Field robots (autonomous agricultural vehicles) are the next logical step in the automation of crop production.

However, safety and liability are the main factors halting their adoption. It is currently unclear whether machinery will continue to grow in size and power or whether crews of smaller robots will conduct certain field operations in the future.

Precision Livestock Management

Several countries, including the member states of the European Union, have established regulations for compulsory electronic identification of cattle, swine, sheep, and goats to prevent the spread of diseases and improve food safety (13). In dairy production, radiofrequency identification (RFID) tags are already used to identify cattle in computer-controlled self-feeders and milking robots (14). Automatic milk feeders for calves customize the milk supplement, measure body weight and body temperature, and generate reports. Milking robots ease the work of dairy operators and allow cows to schedule milking. Additionally, these robots can be adapted to conduct online analysis of milk composition, including cell counts (an important index of hygienic condition), fat, protein, and lactose (15). Knowledge of milk quantity and quality allows for individual feeding of animals (15). Outdoors, GNSS receivers working with other sensors al-

low monitoring of the behavior and well-being of individual animals.

Standardization and Food Traceability

Recently, most of the agricultural industry has agreed to follow the International Standard Organization binary unit system (ISOBUS) as a universal protocol for electronic communication between implements, tractors, and computers (16). ISOBUS ensures data transfer between equipment from different manufacturers, which will allow farmers to control all implements with just one universal onboard computer.

A similar common information exchange protocol is needed to trace the food chain from the farm to the grocery store. This is accomplished using variants of the Extensible Markup Language (XML) [such as agroXML (17)] that allow seamless data interchange between farmers, suppliers, service providers, authorities, processors, and resellers of agricultural produce. Theoretically, this makes it possible to trace back food production to virtually each square meter of a farmer's field. Food traceability and quality control via agroXML have been demonstrated in research projects such as IT FoodTrace (18). Software companies have already started to use agroXML in their information technology products for agriculture and the food industry.

Ultimately, using data feeds regarding production, processing, storage, and retail sale of our foods will enable us to optimize production with minimum waste and cost. Thus, farm managers will not only detect unnecessary treatments but also discover opportunities for boosting production output. Public agencies can obtain data for yield statistics, the calculation of subsidies, and monitoring of the agroecosystem, while they supply farmers with up-to-date information such as the boundaries of water protection areas or the latest pest warnings. Post-harvest industries and food retailers will be able to use various marketing mechanisms to ensure proper supply and quality standards. Together these streams of information will contribute to the main goal of achieving food security in a constantly changing world.

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PERSPECTIVE

African Green Revolution Needn't Be a Mirage

Gebisa Ejeta

Africa missed out on the scientific breakthroughs that revolutionized agriculture in Asia. However, with locally developed and locally relevant technologies, a built-up human and institutional capacity, and supportive national policy and leadership, an African Green Revolution can be a reality.

Sub-Saharan Africa remains the only region in the world where hunger and poverty prevail. In the past 20 years, the number of Africans who live below the global poverty line (\$1 per day) has increased by more than 50%, and more than one-third of the population of the continent continues to suffer from hunger (1). This is of even more concern to African agricultural development as climate change impacts economies largely based on rain-fed agriculture (2). Crop adaptation to climate change requires rigorous research and a multifaceted technological approach that will be much harder to practice on a continent in which agricultural science is in its infancy and the culture of looking to science for solutions to local problems is not well established. However, I believe that Africa has the capacity to feed itself and become a net exporter of food.

In the 1960s, when the Asian Green Revolution was launched, independent Africa was born. Much of the human and institutional capacity essential for an agricultural revolution in Africa was weak or nonexistent. The discoveries of the miracle crop varieties that ignited the Asian Green Revolution were in wheat and rice, two globally important crops, but not in sorghum, millets, maize, or cassava, the critical crops for Africans. That notwithstanding, Africa was not then ready for a science-based development campaign.

Over the next two decades, investments were made from both internal sources and through foreign development assistance for building key institutions, including those of higher education, agricultural research, and technology-transfer institutions such as the agricultural extension services and seed-distributing agencies. Tens of thousands of young African men and women were sent to pursue graduate education in the agricultural sciences at European and North American institutions.

Today, there is a developing, although not yet robust, human capacity base and agricultural research infrastructure focused on seeking solu-

tions for local problems in Africa (3), and links with regional programs and international research centers, foreign universities, and other scientific organizations are supporting this effort. Research collaborations between African scientists and foreign agencies have already yielded important results, for example, the biological control of major insect pests of cassava, the development of rice varieties suitable for Africa, and drought- and parasitic-weed-resistant sorghums. Unfortunately, the growth attained in agricultural education and research (4) has not been matched by a concomitant advance in public and private technology transfer (5) institutions, and results of successful research have not yet been scaled up to the level of the continent.

In addition to the formidable “nature-based” constraints such as prevalence of drought, diverse agro-ecologies, poor soil fertility, and unique pests and diseases, African agricultural development also has to overcome persistent institutional and programmatic challenges. African higher educational institutions still lack the faculty strength and infrastructure to regularly produce high-quality graduates and postgraduates in numbers needed to promote change. Capacity-building and strengthening of local institutions are the areas in which foreign assistance is badly needed.



Fig. 1. Among agricultural pests unique to Africa is the giant witchweed, *Striga hermonthica*. *Striga*'s purple flowers are visible among the damaged sorghum (left). For his work in producing genetically engineered sorghum resistant to the parasite (shown at right), the author was awarded the World Food Prize.

Department of Agronomy, Lilly Hall of Life Sciences, Purdue University, 915 West State Street, West Lafayette, IN 47907–2054, USA. E-mail: gejeta@purdue.edu
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CREDIT: G. EJETA

However, historically, the relationship between Africa and the international aid community has been problematic. Over-reliance on external funding for agricultural development programs has led to a lack of a firm national strategic framework and agendas for national development. Development aid has created unhealthy partnerships with aid recipient national programs made highly susceptible to frequent paradigm shifts generated by foreign agencies.

The earliest paradigm involved the successful basic institution-building programs (6) of the 1950s and 1960s. These programs built human capacity through overseas graduate and undergraduate education in North America and Europe and established new institutions in African countries. They laid the foundations for the needed human and institutional infrastructures, and their contributions have been the most lasting. In the 1970s, a paradigm for technology development and transfer was created, wherein the newly educated cadre was encouraged to start programs for developing and testing modern agricultural practices to take to rural African farms. When programs under this paradigm did not produce immediate impact, they were replaced with programs dubbed farming systems research (7) in the early 1980s. This paradigm argued for scientists to spend time on African farms learning about the realities on farms and in farm households. The 1990s saw the emergence of new paradigms, including programs in sustainable agriculture, participatory plant breeding research (8), and biotechnology, requiring that the agricultural research community pay attention to issues of environmental health, farmer knowledge, and the emergence of new and powerful scientific tools to address the more intractable agricultural problems of rural Africa. In the 2000s, the paradigm of the integrated value-chain approach advocated that research results be adopted to offer productivity gains and advised the needed connections to input and output markets. Although each of these paradigms added new wisdom and built up perspective, the frequent paradigm shifts resulted in changes in funding support that led to a series of failed starts and little progress.

Strengthening human capacity and institutional infrastructure in the essential areas of education, research, and technology transfer depend not only on consistency but also on levels of resource support and choices made. There is enormous variation among public sectors in various African countries and even more on commitments for encouraging the private sector to spur agricultural development (9). In East Africa, Kenya and Uganda have done well in their investments to strengthen their educational institutions and in encouraging private-sector investments. The emergence of a number of small but functional private seed businesses in these two countries is one of the more encouraging

developments in African agriculture in the past several years. Ethiopia has made more substantive and sustained investments in its agricultural research and development enterprise than any other country in Africa. It has developed its agricultural research infrastructure and created a large army of agricultural extension agents. However, Ethiopia has not encouraged the development of its private sector initiatives. It is not the resource capacity of these countries that accounts for the unevenness in the research infrastructure development among them. For example, Nigeria, the wealthiest country in sub-Saharan Africa (other than South Africa) has done an admirable job of building its higher educational institutions but has failed to develop functional infrastructure in agricultural research, extension services, and private-sector input services. In contrast, the poorer countries of Mali in West Africa and Malawi in southern Africa have committed their meager internal resources to building functional institutions and made overt policy statements to encourage private-sector developments. The executive leadership demonstrated by the president of Malawi in promulgating official subsidies to promote the extensive use of improved seeds and inorganic fertilizers to boost farm productivity by the peasant farmers of his country in the past few years has been particularly exemplary.

We are seeing a new sense of urgency and an increased commitment to making a lasting change in African agricultural development (10). We are also seeing an increase in the number and size of institutions engaged in African research and development efforts. One such major initiative that emerged recently in the continent is the Alliance for Green Revolution in Africa (AGRA), an organization created by the joint contributions of the Rockefeller and the Bill and Melinda Gates foundations. AGRA is not a research or development institution; it is a granting agency created to support national agricultural research and development efforts in selected African countries. It would sponsor further development and deployment of technologies generated by existing national agricultural services (NARS) and international agricultural research centers (IARCs). In addition to the IARCs, there are many more foreign institutions engaged in agricultural research in Africa, including several U.S. and European universities. For the eventual goals of AGRA and its national partners to be realized, the allied programs must work well together. What is needed is not a preponderance of independent units operating unengaged with local institutions but a coordinated and mission-oriented program with selective engagement in partnerships leading to proper division of labor and resource commitment.

Despite the challenges, I am optimistic. African leaders have put agriculture on their agendas and made a historic pledge to commit

10% of their national budgets to food security and agriculture-led growth through the Comprehensive Africa Agricultural Development Program (CAADP) (10). Many nations have set a target for science-based annual productivity growth of greater than 6% by 2015. Regional and subregional organizations have been put in place to facilitate technology generation and transfer. Foreign assistance to Africa is being examined and redefined and by various agencies (11); country-led partnerships are given emphasis. Donors at the Group of Eight Summit in 2009 committed more than \$20 billion to support a renewed global effort and invest in comprehensive country-led plans (12). The case for science-based development in Africa may have finally been made.

An African Green Revolution can be a reality, but Africa will not be able to develop a science-based agriculture and economy without considerable external assistance, particularly in the areas of human and institutional capacity building. However, no amount of funding will bring about such a transformative change unless it is locally led by an inspired citizenry and driven by an unequivocal support and commitment from African leaders and policy makers (13).

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PERSPECTIVE

Radically Rethinking Agriculture for the 21st Century

N. V. Fedoroff,^{1*} D. S. Battisti,² R. N. Beachy,³ P. J. M. Cooper,⁴ D. A. Fischhoff,⁵ C. N. Hodges,⁶ V. C. Knauf,⁷ D. Lobell,⁸ B. J. Mazur,⁹ D. Molden,¹⁰ M. P. Reynolds,¹¹ P. C. Ronald,¹² M. W. Rosegrant,¹³ P. A. Sanchez,¹⁴ A. Vonshak,¹⁵ J.-K. Zhu¹⁶

Population growth, arable land and fresh water limits, and climate change have profound implications for the ability of agriculture to meet this century's demands for food, feed, fiber, and fuel while reducing the environmental impact of their production. Success depends on the acceptance and use of contemporary molecular techniques, as well as the increasing development of farming systems that use saline water and integrate nutrient flows.

Population experts anticipate the addition of another roughly 3 billion people to the planet's population by the mid-21st century. However, the amount of arable land has not changed appreciably in more than half a century. It is unlikely to increase much in the future because we are losing it to urbanization, salinization, and desertification as fast as or faster than we are adding it (1). Water scarcity is already a critical concern in parts of the world (2).

Climate change also has important implications for agriculture. The European heat wave of 2003 killed some 30,000 to 50,000 people (3). The average temperature that summer was only about 3.5°C above the average for the last century. The 20 to 36% decrease in the yields of grains and fruits that summer drew little attention. But if the climate scientists are right, summers will be that hot on average by mid-

century, and by 2090 much of the world will be experiencing summers hotter than the hottest summer now on record.

The yields of our most important food, feed, and fiber crops decline precipitously at temperatures much above 30°C (4). Among other reasons, this is because photosynthesis has a temperature optimum in the range of 20° to 25°C for our major temperate crops, and plants develop faster as temperature increases, leaving less time to accumulate the carbohydrates, fats, and proteins that constitute the bulk of fruits and grains (5). Widespread adoption of more effective and sustainable agronomic practices can help buffer crops against warmer and drier environments (6), but it will be increasingly difficult to maintain, much less increase, yields of our current major crops as temperatures rise and drylands expand (7).

Climate change will further affect agriculture as the sea level rises, submerging low-lying cropland, and as glaciers melt, causing river systems to experience shorter and more intense seasonal flows, as well as more flooding (7).

Recent reports on food security emphasize the gains that can be made by bringing existing agronomic and food science technology and know-how to people who do not yet have it (8, 9), as well as by exploring the genetic variability in our existing food crops and developing more ecologically sound farming practices (10). This requires building local educational, technical, and research capacity, food processing capability, storage capacity, and other aspects of agribusiness, as well as rural transportation and water and communications infrastructure. It also necessitates addressing the many trade, subsidy, intellectual property, and regulatory issues that interfere with trade and inhibit the use of technology.

What people are talking about today, both in the private and public research sectors, is the use and improvement of conventional and molecular breeding, as well as molecular genetic modification (GM), to adapt our existing food crops to increasing temperatures, decreased water availability in some places and flooding in others, rising salinity (8, 9), and changing pathogen and

insect threats (11). Another important goal of such research is increasing crops' nitrogen uptake and use efficiency, because nitrogenous compounds in fertilizers are major contributors to waterway eutrophication and greenhouse gas emissions.

There is a critical need to get beyond popular biases against the use of agricultural biotechnology and develop forward-looking regulatory frameworks based on scientific evidence. In 2008, the most recent year for which statistics are available, GM crops were grown on almost 300 million acres in 25 countries, of which 15 were developing countries (12). The world has consumed GM crops for 13 years without incident. The first few GM crops that have been grown very widely, including insect-resistant and herbicide-tolerant corn, cotton, canola, and soybeans, have increased agricultural productivity and farmers' incomes. They have also had environmental and health benefits, such as decreased use of pesticides and herbicides and increased use of no-till farming (13).

Despite the excellent safety and efficacy record of GM crops, regulatory policies remain almost as restrictive as they were when GM crops were first introduced. In the United States, case-by-case review by at least two and sometimes three regulatory agencies (USDA, EPA, and FDA) is still commonly the rule rather than the exception. Perhaps the most detrimental effect of this complex, costly, and time-intensive regulatory apparatus is the virtual exclusion of public-sector researchers from the use of molecular methods to improve crops for farmers. As a result, there are still only a few GM crops, primarily those for which there is a large seed market (12), and the benefits of biotechnology have not been realized for the vast majority of food crops.

What is needed is a serious reevaluation of the existing regulatory framework in the light of accumulated evidence and experience. An authoritative assessment of existing data on GM crop safety is timely and should encompass protein safety, gene stability, acute toxicity, composition, nutritional value, allergenicity, gene flow, and effects on nontarget organisms. This would establish a foundation for reducing the complexity of the regulatory process without affecting the integrity of the safety assessment. Such an evolution of the regulatory process in the United States would be a welcome precedent globally.

It is also critically important to develop a public facility within the USDA with the mission of conducting the requisite safety testing of GM crops developed in the public sector. This would make it possible for university and other public-sector researchers to use contemporary molecular knowledge and techniques to improve local crops for farmers.

However, it is not at all a foregone conclusion that our current crops can be pushed to perform as well as they do now at much higher temperatures and with much less water and other agricultural inputs. It will take new approaches, new methods,

¹Office of the Science and Technology Adviser to the Secretary of State and to the Administrator of USAID, U.S. Department of State, Washington, DC 20520, USA. ²Department of Atmospheric Sciences, University of Washington, Seattle, WA 98195, USA. ³National Institute of Food and Agriculture, U.S. Department of Agriculture, Washington, DC 20250, USA. ⁴International Crops Research Institute for the Semi-Arid Tropics, Nairobi, Kenya. ⁵Monsanto Company, St. Louis, MO 63167, USA. ⁶Seawater Foundation, Tucson, AZ 85711, USA. ⁷Arcadia Biosciences, Davis, CA 95618, USA. ⁸Department of Environmental Earth System Science and Program on Food Security and the Environment, Stanford University, Stanford, CA 94305, USA. ⁹DuPont Agriculture & Nutrition, DuPont Experimental Station, 200 Powder Mill Road, Wilmington, DE 19805, USA. ¹⁰International Water Management Institute, 127 Sunil Mawatha, Pelawatte, Battaramulla, Colombo, Sri Lanka. ¹¹International Maize and Wheat Improvement Center, Km. 45, Carretera Mexico-Veracruz, El Batán, Texcoco, Edo. de México, CP 56130, México. ¹²Department of Plant Pathology, University of California, Davis, CA 95616, USA, and Joint Bioenergy Institute, Emeryville, CA 94608, USA. ¹³International Food Policy Research Institute, Washington, DC 20006, USA. ¹⁴Earth Institute, Columbia University, Palisades, NY 10964, USA. ¹⁵Blaustein Institutes for Desert Research, Ben Gurion University, Sede Boqer Campus, Sede Boqer 84990, Israel. ¹⁶Center for Plant Stress Genomics and Technology, King Abdullah University of Science and Technology, Kingdom of Saudi Arabia, and Department of Botany and Plant Sciences, University of California, Riverside, CA 92521, USA.

*To whom correspondence should be addressed. E-mail: fedoroff@state.gov



Fig. 1. Saline farming. Upper and lower right, brackish-water agriculture and tomato farming, Negev desert, Israel; center, saline farming of the halophyte salicornia, Eritrea.

new technology—indeed, perhaps even new crops and new agricultural systems.

Aquaculture is part of the answer. A kilogram of fish can be produced in as little as 50 liters of water (14), although the total water requirements depend on the feed source. Feed is now commonly derived from wild-caught fish, increasing pressure on marine fisheries. As well, much of the growing aquaculture industry is a source of nutrient pollution of coastal waters, but self-contained and isolated systems are increasingly used to buffer aquaculture from pathogens and minimize its impact on the environment (15).

Another part of the answer is in the scale-up of dryland and saline agriculture (Fig. 1) (16). Among the research leaders are several centers of the Consultative Group on International Agricultural Research, the International Center for Biosaline Agriculture, and the Jacob Blaustein Institutes for Desert Research of the Ben-Gurion University of the Negev.

Systems that integrate agriculture and aquaculture are rapidly developing in scope and sophistica-

tion. A 2001 United Nations Food and Agriculture Organization report (17) describes the development of such systems in many Asian countries. Today, such systems increasingly integrate organisms from multiple trophic levels (18). An approach particularly well suited for coastal deserts includes inland seawater ponds that support aquaculture, the nutrient efflux from which fertilizes the growth of halophytes, seaweed, salt-tolerant grasses, and mangroves useful for animal feed, human food, and biofuels, and as carbon sinks (19). Such integrated systems can eliminate today's flow of agricultural nutrients from land to sea. If done on a sufficient scale, inland seawater systems could also compensate for rising sea levels.

The heart of new agricultural paradigms for a hotter and more populous world must be systems that close the loop of nutrient flows from micro-organisms and plants to animals and back, powered and irrigated as much as possible by sunlight and seawater. This has the potential to decrease the land, energy, and freshwater demands of agriculture, while at the same time ameliorating

the pollution currently associated with agricultural chemicals and animal waste. The design and large-scale implementation of farms based on nontraditional species in arid places will undoubtedly pose new research, engineering, monitoring, and regulatory challenges, with respect to food safety and ecological impacts as well as control of pests and pathogens. But if we are to resume progress toward eliminating hunger, we must scale up and further build on the innovative approaches already under development, and we must do so immediately.

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Prdm9 Controls Activation of Mammalian Recombination Hotspots

Emil D. Parvanov, Petko M. Petkov,* Kenneth Paigen*

Genetic recombination is an essential biological process among eukaryotes. Mammalian meiotic recombination, which preferentially occurs at specialized sites, 1 to 2 kb long, known as hotspots, assures the orderly segregation of meiotic chromosomes and creates genetic variation among offspring. However, despite their importance in shaping the recombination landscape of the organism, we have little understanding of the elements determining location and relative activity of hotspots. That the location of a hotspot is not determined simply by its internal DNA sequence is supported by both yeast (1) and mammalian studies (2, 3). Two recent reports (4, 5) have described the existence of trans-acting loci that control the activation of specific hotspots elsewhere. The loci, *Dsbc1* and *Rcr1*, are located in overlapping 5.4-Mb and 6.3-Mb regions on mouse chromosome 17.

We have now extended the mapping of both *Rcr1* and *Dsbc1* by using 1580 male progeny of a cross between the C57BL/6J (B6) and CAST/EiJ (CAST) mouse strains differing in activity of the *Rcr1/Dsbc1* controlled hotspots *Hlx1*, *Esrrg-1*, and *Psmb9* (fig. S1). We located both loci to a common 181-kb region containing four protein-coding genes (Fig. 1): *Psmb1*, *Tbp*, *Pdcd2*, and *Prdm9*. We found no differences in expression of the transcripts of any of these proteins in testes when assayed by reverse transcription polymerase chain reaction (RT-PCR) (table S1).

Psmb1, proteasome beta type 1 subunit, is a ubiquitously expressed protein that functions as a structural component of an organelle responsible for generalized protein degradation. *Tbp*, TATA binding protein, is an essential component of the TFIID transcription initiation complex, which has considerable DNA binding specificity. *Pdcd2*, programmed cell death protein 2, is widely expressed and repressed during B cell lymphomagenesis. On the basis of DNA sequencing of the entire coding regions of these three proteins, we found only five coding single-nucleotide polymorphisms (SNPs), all synonymous, resulting in no amino acid differences between the B6 and CAST strains (table S2). For the first exon of *Pdcd2*, we relied on the Sanger Institute sequence (www.sanger.ac.uk/modelorg/mousegenomes/).

Prdm9, PR domain containing 9, is uniquely expressed during early meiosis in both males and females, and the knockout is blocked at the pachytene stage of meiosis I with reduction of the num-

ber of *Dmc1* loci and a loss of *Dmc1* and γ -H2AX colocalization, resulting in sterility in both sexes and azoospermia in males (6). It has three functional domains, a N-terminal KRAB domain that can promote protein-protein binding and transcriptional repression when tethered to DNA by an

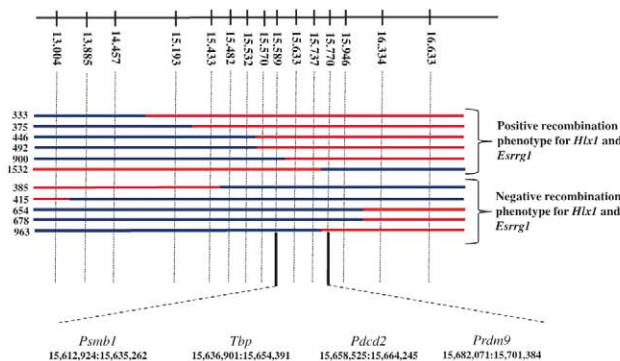


Fig. 1. Fine-mapping the location of *Rcr1*. Upper line shows the position, in Mb (National Center for Biotechnology Information Build 37), of the markers used for genetic mapping. Crossovers collected in this region are marked with blue for B6 DNA and red for CAST. On the left are the serial numbers of the male progeny tested for recombination activity. The critical region containing *Rcr1* is between markers at 15.589 and 15.770 Mb. Below are the order and coordinates of the four protein-coding genes in the critical region.

adjacent DNA binding domain, a central PR/SET domain providing a histone methyl transferase activity capable of trimethylating H3K4 and thus altering chromatin configuration, and a terminal zinc finger domain of C2H2 type. The zinc finger domain contains tandem repeats, with one finger per repeat, and has 11 fingers in the CAST allele and 12 in the B6 allele, with a number of amino acid substitutions in the 6, 9, and 12 positions after the second cysteine of the fingers, the residues determining DNA binding specificity (fig. S2). Given the properties of *Prdm9*, including its requirement for meiosis, its histone 3 lysine-4 trimethylation activity and role in determining DNA double-stranded breaks (DSBs), and the lack of a reasonable alternative candidate, we conclude that *Prdm9* is *Rcr1/Dsbc1*. This is supported by the observation (7) that recombination at hotspots *Psmb9* and *Hlx1*, regulated by *Rcr1/Dsbc1*, is preceded by histone 3 lysine-4 trimethylation.

We sequenced *Prdm9* exon 12 containing the entire Zn finger domain in 20 mouse strains and found five alleles differing in the number of zinc finger repeats, ranging from 11 to 14, as well as in codons for the amino acids in sixth, ninth, and 12th position of each finger responsible for DNA binding (fig. S2). The alleles for C3H and PWD mouse strains are identical with those reported by Mihola *et al.* (8), who identified *Prdm9* as the

gene underlying the *Hst1* locus determining male infertility in certain crosses between strains of *M. musculus* and *M. domesticus*.

We also sequenced exon 12 of human *PRDM9* in 64 DNA samples from the Coriell Institute for Medical Research. We found two predominant alleles among these 128 chromosomes, containing a zinc finger domain of 12 tandem repeats and several minor frequency alleles (fig. S3A). African-Americans had 11 minor alleles with 11, 12, or 13 zinc fingers and a number of amino acid substitutions; Han Chinese three minor alleles, two with 12 and one with 13 fingers; and Mexican-Americans one, identical to the Chinese variant with 13 fingers; whereas Caucasians had none (fig. S3B). These results conform well with presently accepted views of human evolution.

The identification of *Prdm9* as a mammalian protein regulating meiotic recombination hotspots initiates studies of an important biological control system that has hitherto been inaccessible. A recent paper (9) identified *Prdm9* as a speciation gene across diverse metazoans and hypothesized that its essential role in meiosis is directly related to its ability to bind rapidly evolving DNA sequences. Our results show that these sequences represent recombination hotspots. An immediate compelling question is whether *Prdm9* controls activation of all, or nearly all, recombination hotspots, or whether it is simply a member of a family of proteins, each controlling a subset of all hotspots.

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Supporting Online Material

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Table S1
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The Jackson Laboratory, Bar Harbor, ME 04609, USA.

*To whom correspondence should be addressed. E-mail: Petko.Petkov@jax.org (P.M.P.); Ken.Paigen@jax.org (K.P.)

PRDM9 Is a Major Determinant of Meiotic Recombination Hotspots in Humans and Mice

F. Baudat,^{1*} J. Buard,^{1*} C. Grey,^{1*} A. Fledel-Alon,² C. Ober,² M. Przeworski,^{2,3} G. Coop,⁴ B. de Massy^{1†}

Meiotic recombination events cluster into narrow segments of the genome, defined as hotspots. Here, we demonstrate that a major player for hotspot specification is the *Prdm9* gene. First, two mouse strains that differ in hotspot usage are polymorphic for the zinc finger DNA binding array of PRDM9. Second, the human consensus PRDM9 allele is predicted to recognize the 13-mer motif enriched at human hotspots; this DNA binding specificity is verified by in vitro studies. Third, allelic variants of PRDM9 zinc fingers are significantly associated with variability in genome-wide hotspot usage among humans. Our results provide a molecular basis for the distribution of meiotic recombination in mammals, in which the binding of PRDM9 to specific DNA sequences targets the initiation of recombination at specific locations in the genome.

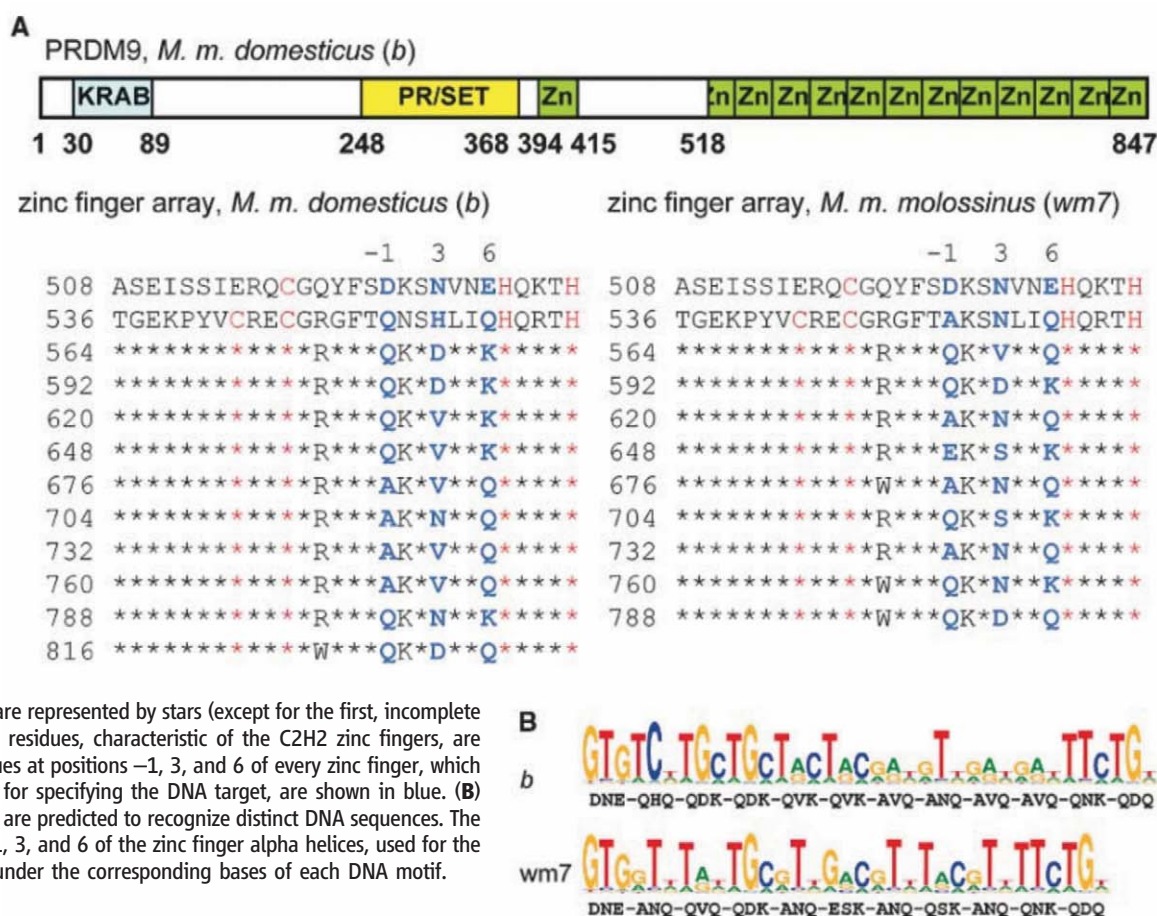
Meiosis is a specialized cell cycle, essential for sexual reproduction, in which diploid cells give rise to haploid gametes. The halving of genome content during meiosis results from two successive divisions. During the first one, the reductional division, which is unique to meiotic cells, homologous chromosomes segregate. This segre-

gation requires the establishment of connections between homologs that are mediated in most species by reciprocal recombination events known as crossing over (CO) (1). COs also increase genome diversity, thereby improving the efficacy of natural selection (2). The molecular process of CO formation involves a highly regulated pathway of induction of programmed

DNA double-strand breaks (DSBs), followed by their repair on the homolog (3). In yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, initiation sites have been mapped by the direct molecular detection of DSBs. These studies have shown that DSBs are not randomly distributed along chromosomes but occur in specific regions of the genome, according to rules that are as yet poorly understood (4). A common chromatin feature, the trimethylation of lysine 4 of histone H3 (H3K4me3), defines yeast and mouse initiation sites (5, 6).

In mammals, in most cases, the locations of initiation sites are deduced from mapping CO events. COs can be mapped at high resolution, by pedigree analysis, detection of recombinant molecules in gametes, or analysis of linkage disequilibrium (LD) (7, 8). In humans, these approaches have shown that most COs are clustered in narrow regions (1 to 2 kb), called hotspots, that are predicted to be preferred initiation sites

Fig. 1. Mouse *wm7* and *b* *Prdm9* alleles are polymorphic at residues involved in specifying DNA targets in the zinc finger array. (A) Tandem repeat structure of the mouse PRDM9 zinc finger array. (Top) The structure of the mouse *b* allele is shown, with the Krüppel-associated box (KRAB), the PR/SET domain, and the zinc fingers (Zn) shaded in blue, yellow, and green, respectively. (Bottom) Sequences of the C-terminal tandem arrays of zinc fingers of the *b* allele (left) and the *wm7* allele (right). The coordinate of the first residue of each repeat on the protein sequence is indicated. The residues identical to the second repeat are represented by stars (except for the first, incomplete zinc finger). The C and H residues, characteristic of the C2H2 zinc fingers, are depicted in red. The residues at positions -1, 3, and 6 of every zinc finger, which are of special importance for specifying the DNA target, are shown in blue. (B) PRDM9 *wm7* and *b* alleles are predicted to recognize distinct DNA sequences. The amino acids at position -1, 3, and 6 of the zinc finger alpha helices, used for the prediction, are indicated under the corresponding bases of each DNA motif.



¹Institut de Génétique Humaine, UPR1142, CNRS, Montpellier, France. ²Department of Human Genetics, University of Chicago, Chicago, IL 60637, USA. ³Howard Hughes Medical Institute, University of Chicago, Chicago, IL 60637, USA. ⁴Department of Evolution and Ecology and the Center for Population Biology, University of California, Davis, CA 95616, USA.

*These authors contributed equally to this work.

†To whom correspondence should be addressed. E-mail: bernard.de-massy@igh.cnrs.fr

(9). On the basis of LD patterns, more than 30,000 hotspots have been identified in the human genome, spaced, on average, every 50 to 100 kb, often outside from genes and with highly variable levels of activity (10, 11). In addition, some hotspots show interindividual variation in activity as shown by sperm-typing studies (7) or pedigree analysis (12).

LD-based hotspots were found to be highly enriched for a degenerate 13-mer motif (13). Moreover, in sperm-typing studies, single-nucleotide polymorphisms within this 13-base pair (bp) motif were found to be associated with variation of hotspot activity in cis (14, 15). Genome-wide, the motif plays a role in ~40% of hotspots and is proposed to be involved in initiation specification or other aspects of recombination activity (13). In mice, on the basis of the analysis of a 25-Mb interval on chromosome (chr) 1 (16) and several individual regions (17), initiation of meiotic re-

combination also appears to be clustered in small intervals. Recently, by comparing recombination activity between different mouse strains, a genetic locus responsible for the distribution of recombination in the genome was identified (18, 19), which potentially contributes, either directly or indirectly, to the specification of initiation sites in the genome. Specifically, the genetic background at this locus (*w*m7 haplotype from *Mus musculus molossinus* or *b* haplotype from *M. m. domesticus* strains C57BL/10 or C57BL/6) was found to affect recombination activity measured both chromosome-wide and at two individual hotspots (*Psmb9* on chr 17 and *Hlx1* on chr 1). This locus (named *Dsbc1*) was mapped to a region between 10.1 and 16.8 Mb on mouse chr 17 (18).

Prdm9, a candidate gene. Upon additional crossing, we refined the *Dsbc1* locus to the 12.2- to 16.8-Mb region of mouse chr 17 [see supporting online material (SOM) text]. This region con-

tains the *Prdm9* gene coding for a protein with a PRD1-BF1 and RIZ/Su(var)3-9, enhancer-of-zeste, and trithorax (PRSET)-methyl transferase domain and a tandem array of 12 C2-H2 zinc fingers. PRDM9 has been shown to trimethylate H3K4 and is expressed specifically in germ cells during meiotic prophase (20). Strains with distinct *Dsbc1* alleles (*wm7* or *b*) have different levels of H3K4me3 at the two recombination hotspots, *Psmb9* and *Hlx1*. Specifically, a high level of H3K4me3 was correlated with high recombination activity at these hotspots (6). The *Prdm9* gene is the only reported gene encoding for a histone methyl transferase in the *Dsbc1* region and thus represents a strong candidate gene for the effect of *Dsbc1*. On this basis, we reasoned that the zinc fingers of PRDM9 could mediate DNA binding specificity and thus target its activity to specific sites in the genome. According to this hypothesis, altering the zinc fingers is predicted to lead to changes of sites targeted by PRDM9.

Distinct predicted DNA sequence specificities for two mouse PRDM9 zinc finger variants.

We therefore determined and compared the cDNA sequences of *Prdm9* from *M. m. molossinus* (*wm7*) and *M. m. domesticus* (*b*) (Fig. 1A and fig. S1). These two *Prdm9* alleles showed a high level of polymorphism (24 changes over 847 residues); all but one of the changes are located in the zinc finger array. This array, encoded within a single exon, has a minisatellite-like genomic structure in which each zinc finger, 28 amino acids long, is encoded within a 84-bp unit, which is repeated in tandem with almost perfect homology at both the DNA and protein levels (fig. S1). For a given allele, the differences between repeats are restricted to seven positions, five of which encode for amino acids at coordinates -1, 3, and 6 of the zinc finger alpha helix, predicted to be in contact with the DNA and known to be involved in DNA sequence specificity (21, 22). When comparing the two *Prdm9* alleles (*wm7* and *b*), most polymorphisms (21 out of 23) were at residues -1, 3, and 6 of the zinc finger (Fig. 1A and fig. S1). The *wm7* allele is also missing one zinc finger compared with the *b* allele. Sequencing the *Prdm9* zinc finger array from *M. m. castaneus* showed it to be identical to *wm7*. This is consistent with the genetic origin of *M. m. molossinus*, known to be in part derived from *M. m. castaneus*, and with the observation that the two hotspots, *Psmb9* and *Hlx1*, are active at similar levels in the presence of *Dsbc1* alleles from either *M. m. castaneus* or *M. m. molossinus* [(18) and SOM text]. Using the Zinc Finger Consortium Database (23, 24), we predict that these two PRDM9 proteins preferentially recognize distinct DNA motifs (Fig. 1B). Due to the low predicted specificity of some zinc fingers and the multiple combinations through which several zinc fingers of a protein may contribute to DNA recognition, PRDM9 is expected to recognize a large number of sites in the genome. For these reasons, and also because of the limited DNA recognition predictability of some zinc fingers (25), the predicted motif has limited power in

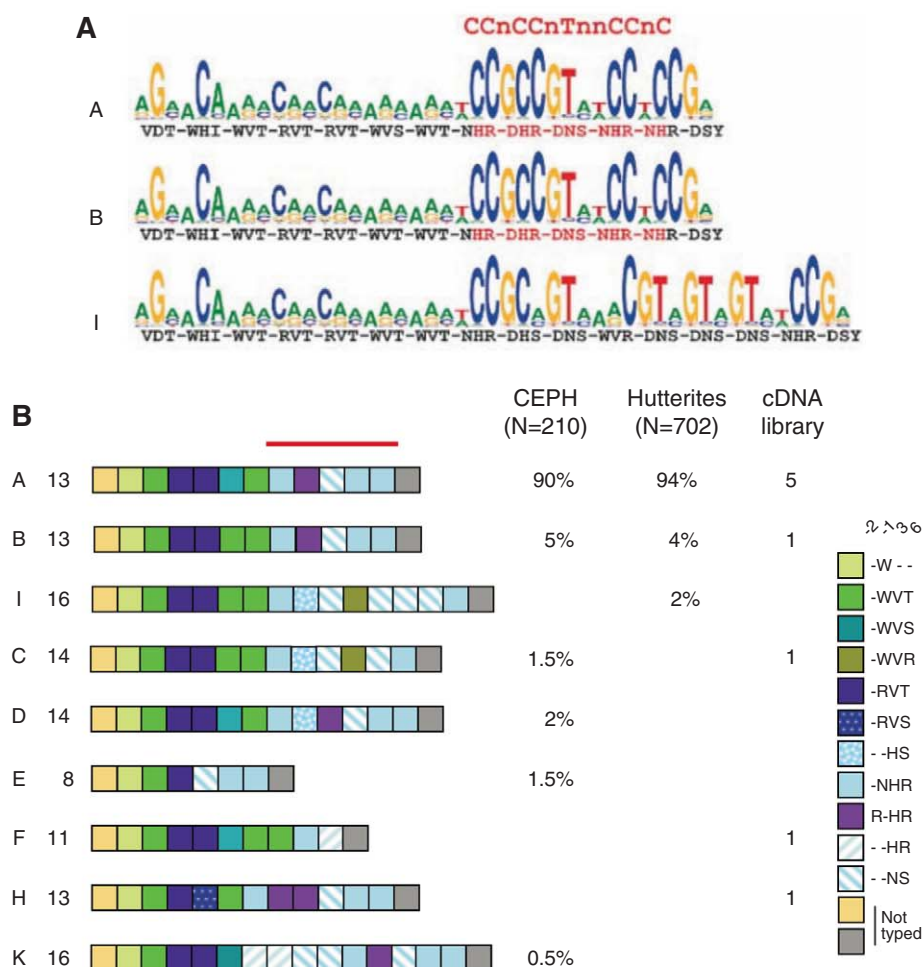


Fig. 2. (A) Human PRDM9 major alleles (alleles A and B) are predicted to bind the 13-mer hotspot motif, whereas the I allele is predicted to bind a distinct motif. The LD-based hotspot consensus identified by Myers *et al.* (13) is shown above. The amino acids at position -1, 3, and 6 of the zinc finger alpha helices are indicated as in Fig. 1B, with the residues predicted to recognize the LD-hotspot consensus motif shown in red. **(B)** Allelic diversity of the human PRDM9 zinc finger tandem array. Interspersion patterns of variant repeats (colored boxes) of alleles from unrelated individuals were established by either MVR mapping (105 CEPH unrelated parents or grandparents and 351 Hutterite parents) or sequencing clones from a testis cDNA library made from 39 donors. Major allele A and minor allele B were found in all three sets of unrelated individuals; other rare alleles were only found in one or two sets. The structures of some rare alleles (I, C, E, and F) differ strongly from alleles A and B in the region encoding the critical domain (red bar) for recognition of the 13-mer hotspot motif. N, number of alleles.

identifying PRDM9 binding sites. Nonetheless, it is noteworthy that sequences respectively matching 8 and 9 of the 13 highest score bases of PRDM9^{wm7} predicted recognition motif are found near the center of *Psmb9* and *Hlx1* hotspots (fig. S2).

Variability in human PRDM9 zinc fingers. In humans, the degenerate 13-mer motif was proposed to be a potential binding site for zinc fingers, given its apparent 3-bp periodicity (13). Therefore, we analyzed the zinc finger region of the human PRDM9 protein for its predicted binding specificity. The human PRDM9 protein referenced in databases (Ensembl release 56, based on the Genome Reference Consortium GRCh37) contains 13 zinc fingers, with a tandem repeat structure similar to that observed in mice, in which repeats are highly identical except at positions -1, 3, and 6 of the zinc finger alpha helices (fig. S3A). Notably, a group of five zinc fingers had a predicted affinity for a sequence that matches the 13-mer hotspot motif (Fig. 2A). This finding suggested to us that the role for *Prdm9* in specifying hotspot localization might be conserved from mouse to human. If so, we might expect allelic variation in the zinc finger array to be associated with hotspot usage differences among humans. To test these predictions, we analyzed *Prdm9* polymorphism by sequencing individual cDNAs from a testis library derived from a pool of 39 individuals and also by genotyping the zinc finger array by minisatellite variant repeat (MVR)-PCR (26) in individuals of European ancestry: the Centre d'Etude du Polymorphisme Humain (CEPH) resources and the Hutterites, a founder population currently living in North America (Fig. 2B and figs. S3B and S4). A large number of alleles were found with differences in both the number of repeats and their identity. In the CEPH families, six alleles were found among 105 unrelated individuals, with the major allele (allele A) occurring at a frequency of 90%. Except for one amino acid change in the 6th zinc finger, allele A is identical to the genome sequence reference allele (allele B), which is at a frequency of 5%. Among other alleles (named C, D, E, and K), the first five zinc fingers of PRDM9 show little variability, but zinc fingers 8 to 11 from allele A are highly variable with amino acid changes at the positions involved in contact with the DNA (fig. S5). Variability in humans seems to be concentrated on one side of the zinc finger array, in the region involved in recognition of the 13-mer motif in allele A.

Association of human PRDM9 zinc finger variants with hotspot usage. In the Hutterite sample (26), three *Prdm9* alleles, A, B, and I, were present at frequencies of 94, 4, and 2%, respectively. Given the amino acid changes in its zinc finger array, the I allele variant is not expected to recognize the 13-mer motif (Fig. 2A). The presence of these variants allowed us to test the functional relation between *Prdm9* alleles, their predicted binding specificity, and hotspot usage, taking advantage of well-localized CO events in Hutterite families. Variation among Hutterite parents with respect to genome-wide

“hotspot usage” (the fraction of COs that occurred in recombination hotspots inferred from LD data) was previously found to be significant and heritable [$h^2 = 0.22$ (12)]. To increase our sample size, we typed an additional 188 Hutterite parents, in which we found 6 AI and 10 AB genotypes. Among these, we were able to call crossover events in transmissions from an additional two AB individuals, three AI individuals, and their five AA partners (i.e., the subset of parents for which genotyping information was available for two or more children). To assess the impact of variation at the zinc finger array of *Prdm9* on hotspot usage in the Hutterites, we regressed the maximum likelihood estimate of hotspot usage for each parent on his/her genotype (Fig. 3A). Both AB and AI heterozygote individuals differed significantly from AA homozygotes in their use of LD-based hotspots of recombination ($P_{AB} = 0.033$, $P_{AI} = 9.3 \times 10^{-12}$). The AI heterozygotes had significantly lower hotspot usage in both males

and females ($P_{AI} = 1.6 \times 10^{-8}$ and $P_{AI} = 0.0032$, with $n_{AI} = 7$ and $n_{AI} = 2$, respectively, where n is the number of individuals), whereas the AB result was only significant in females ($P_{AB} = 0.020$, $n_{AB} = 9$), but was in a consistent direction in males ($P_{AB} = 0.40$, $n_{AB} = 9$). This result was robust to the relatedness among Hutterite individuals and remained significant when the phenotypes were quantile normalized (26). Moreover, variation at the zinc finger array of *Prdm9* alone explained 18% of the population variance in hotspot usage among Hutterite individuals (26); the true proportion is likely to be even higher, given that the phenotype is measured with considerable error.

Because individuals differ greatly in the precision with which their phenotype is estimated due to differences in the number of well-localized CO events (Fig. 3A), we considered whether this measurement error could affect our conclusions. To this end, we calculated the likelihood surface for the hotspot usage phenotype for each geno-

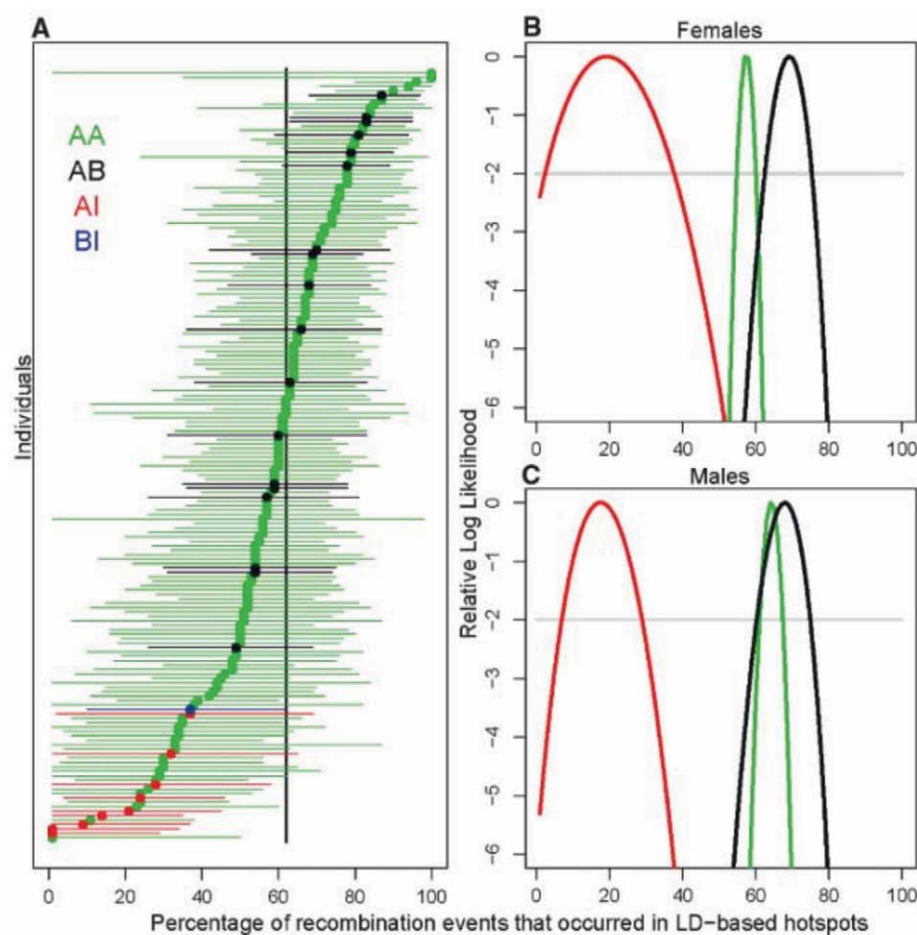


Fig. 3. Association of human *Prdm9* alleles with genome-wide (LD-based) hotspot usage. The different genotypes for variants in the zinc finger array are indicated by different colors. (A) In each individual, the percentage of recombination events that occurred in LD-based hotspots. The maximum likelihood estimate (MLE) for each individual is shown as a point, and the 95% confidence intervals (asymptotic cutoff) are indicated by the lengths of the horizontal lines. Individuals are ordered by their MLE values. The black vertical line shows the joint MLE for all individuals. (B and C) The relative log likelihood surfaces of the percentage of recombination events that occurred in LD-based hotspots for the three genotypes (AA, AB, and AI) in females and males, respectively. The curve for the BI genotype is left out because of low sample size ($n = 1$). The gray horizontal line is provided as a visual guide, to indicate where the asymptotic cutoff is for the 95% confidence interval.

type, in females and males (Fig. 3, B and C). A likelihood ratio test of a model in which hotspot usage does not depend on genotype to one in which it does was highly significant in both males and females [$P = 0.0014$ in females, $P < 10^{-5}$ in males, as assessed by permutation (26)]. Notably, the AI genotype is associated with a threefold ($\sim 70\%$) drop in the usage of LD-based hotspots (the maximum likelihood estimates fall from 60 to 18% in the joint analysis of males and females, see fig. S6). The large difference in LD-based hotspot usage between AA and AI individuals suggests that the I allele activates a set of hotspots that have not left a footprint on genetic diversity, either because they are too recent or too weak. The interpretation of the difference in hotspot usage between AA and AI individuals depends on how many crossovers are specified by the A allele in AA individuals. As a first approximation, we might consider that the 13-mer motif has been predicted to be causal at 40% of LD hotspots (13) and, thus, all else being equal, that 40% of crossovers placed in LD hotspots might depend on the A allele. The fact that the estimated difference between genotypes is far larger ($\sim 70\%$) suggests that the binding specificity of PRDM9 explains more than 40% of LD-based hotspot activity in the current population. In any case, the strong decrease observed in AI heterozygotes suggests that the I allele is out-competing the A allele in determining crossovers in LD-based hotspots, for example, because of a greater number of sites recognized or a higher binding affinity. The small but significant increase in LD-based hotspot usage in AB compared with AA individuals suggests that the sequences recognized by A and B are slightly different. This might be explained by the amino acid difference (serine to threonine) between these two alleles (Fig. 2A), located on a residue of a zinc finger potentially involved in interaction with the DNA.

Furthermore, whereas across individuals, hotspot usage was not significantly correlated with genetic map length (12), AB heterozygotes showed a significantly longer genetic map in the combined sample of both sexes ($P_{AB} = 0.014$). This effect remained, even when the phenotype was quantile normalized. In contrast, there was no detectable effect of the AI heterozygote on the map length ($P_{AI} = 0.37$) (26).

Direct PRDM9 binding to hotspot motifs.

Together, these results provide direct evidence that *Prdm9* is involved in hotspot specification and in controlling the distribution of recombination events in the human genome. To demonstrate that this effect is mediated through the binding of PRDM9 at hotspots, we directly tested the interaction between PRDM9^A and PRDM9^I proteins and their predicted recognition motifs. By southwestern analysis, PRDM9^A protein (labeled ZA) was shown to have high affinity to a DNA fragment including the 13-mer hotspot motif (HM); it was also found to have low affinity to the same fragment carrying mutations in the most conserved positions of this motif (HM*), as well as to a DNA fragment including the predicted motif of the PRDM9^I protein (IM) (Fig. 4, A to C). Reciprocally, binding of PRDM9^I (ZI) was specific for the predicted I motif (Fig. 4B). These assays were independently confirmed by band-shift assays that showed the greater affinity of PRDM9^A to the 13-mer hotspot motif compared with its mutated form and to the predicted I motif, as well as the greater affinity of the PRDM9^I for the predicted I motif compared with the 13-mer hotspot motif (Fig. 4D).

In summary, our observations reveal an entirely unexpected feature of initiation of meiotic recombination: a role for *Prdm9* in specifying the sites of initiation in mammals, through the direct binding of PRDM9 to specific sequences

in the genome and by promoting DSB formation in the vicinity of its binding site. Using a different strategy, Myers *et al.* (27) predicted the preferential binding of human PRDM9 to the 13-mer hotspot motif, and thus proposed PRDM9 to be involved in hotspot localization in humans. The precise mechanism of action of *Prdm9* is not known. It is likely that the histone methyl transferase activity has an important role by promoting enrichment of H3K4me3 on nucleosomes located next to PRDM9 binding sites as observed at two mouse hotspots (6). In turn, this modification of the chromatin, or downstream signals, might be recognized by a component of the recombination initiation machinery allowing the recruitment of SPO11 that catalyzes meiotic DSB formation. Interestingly, in *S. cerevisiae*, the enrichment for H3K4me3 has also been observed at initiation sites (5). In this case, this histone modification depends on the histone methyl transferase Set1 that does not contain a DNA binding domain and that is probably recruited by an alternative mechanism. In mice and humans, PRDM9 seems to control the activity of a large fraction of hotspots. In fact, the presence of different *Prdm9* alleles leads to major changes of crossover distribution on several chromosomes in mice (18, 19) and substantial changes in hotspot usage in humans (Fig. 3). Analysis of *Prdm9*⁺ mice has shown that *Prdm9* is essential for progression through meiotic prophase (20). On the basis of cytological analysis, DSBs were detected in *Prdm9*⁺ spermatocytes, suggesting that *Prdm9* might not be absolutely required for DSB formation. It is therefore possible that in the wild-type, some DSBs might occur at sites not bound by PRDM9.

Prdm9 has also been shown to be involved in hybrid sterility in *M. musculus*. This phenotype depends on polymorphisms in the zinc finger array of PRDM9 and on several independently

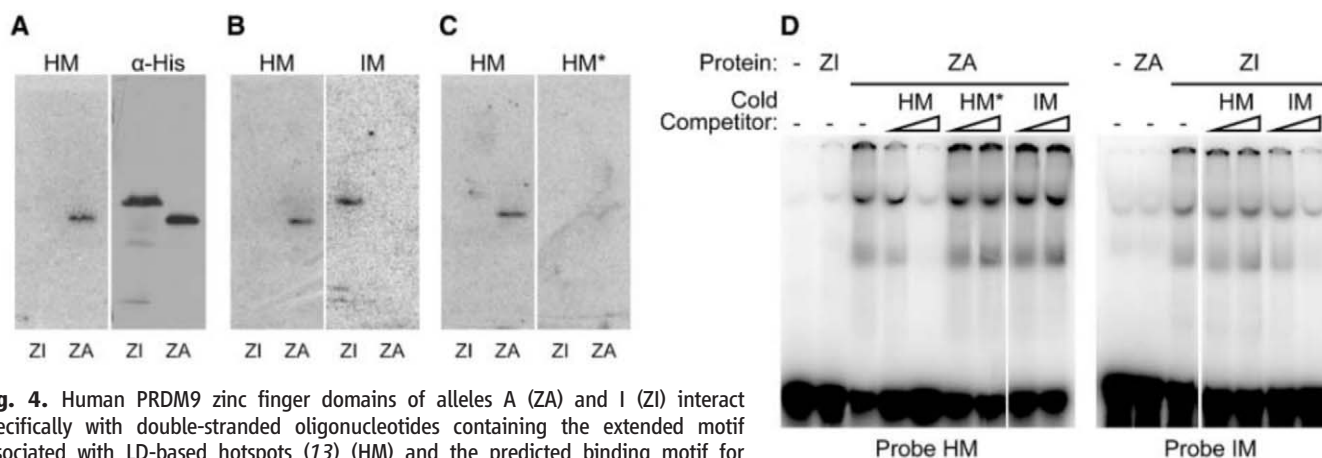


Fig. 4. Human PRDM9 zinc finger domains of alleles A (ZA) and I (ZI) interact specifically with double-stranded oligonucleotides containing the extended motif associated with LD-based hotspots (13) (HM) and the predicted binding motif for hPRDM9 I allele (IM), respectively. (A to C) (Left panels) Southwestern blotting experiment performed with His-tagged ZI and ZA proteins from total *E. coli* extracts, probed with HM. (Right panels) Mirror-image blots obtained after diffusion transfer to a membrane placed on the other side of the same protein gel (26). (A) Immunoblotting experiment using monoclonal α -polyhistidine antibody. (B) Southwestern blotting using the IM probe. (C) Southwestern blotting using the HM* probe, which contains multiple mutations in the 13-mer motif. (D) Electrophoretic mobility shift assays with in vitro translated glutathione S-transferase-hPRDM9 zinc finger domain fusions of alleles A (ZA) or I (ZI). The probes on the left and right panels are HM and IM, respectively. Cold competitor, in molar excess of 20- and 200-fold over the probe, has been added as mentioned.

segregating genes (28). In sterile hybrids, a defect is observed during meiotic prophase, after the stage of DSB formation, which may indicate an additional role for PRDM9 (for instance, in the regulation of gene expression) and presumably involves a limited number of genes. In fact, one does not expect PRDM9 to be a master transcriptional regulator given the rapid evolution of its DNA binding specificity among metazoans (29).

The features of the PRDM9 protein described above carry major implications for hotspot variability and genome evolution. The minisatellite structure of the *Prdm9* zinc finger encoding region confers a strong potential to generate variability by recombination or replication slippage within the array. Specifically, a single-amino acid change within zinc fingers could lead to a PRDM9 variant with novel DNA binding specificity and, thus, could potentially create a new family of hotspots genome-wide. The introduction of new hotspots may counteract the loss of individual hotspots due to biased gene conversion upon DSB repair (which acts against the initiating allele), and so changes in the *Prdm9* gene offer a mechanistic solution to the "recombination hotspot paradox" (30). Rapid evolution of both the PRDM9 protein and the hotspot motif have been shown by Myers *et al.* (27). Further, the zinc fingers of PRDM9 are evolving under positive selection and concerted evolution across many metazoan species, specifically at positions involved in defining their DNA-binding specificity (29). Regardless of the precise selective pressures acting on this gene, the properties of PRDM9 uncovered here, together with features of DSB repair, provide an interpretation for the divergence of fine-scale genetic maps between closely related spe-

cies and even among individuals within species (19, 31, 32).

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Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1183439/DC1

Materials and Methods

SOM Text

Figs. S1 to S7

Table S1

References

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REPORTS

Resonance Fluorescence of a Single Artificial Atom

O. Astafiev,^{1,2*} A. M. Zagoskin,³ A. A. Abdumalikov Jr.,^{2†} Yu. A. Pashkin,^{1,2‡} T. Yamamoto,^{1,2} K. Inomata,² Y. Nakamura,^{1,2} J. S. Tsai^{1,2}

An atom in open space can be detected by means of resonant absorption and reemission of electromagnetic waves, known as resonance fluorescence, which is a fundamental phenomenon of quantum optics. We report on the observation of scattering of propagating waves by a single artificial atom. The behavior of the artificial atom, a superconducting macroscopic two-level system, is in a quantitative agreement with the predictions of quantum optics for a pointlike scatterer interacting with the electromagnetic field in one-dimensional open space. The strong atom-field interaction as revealed in a high degree of extinction of propagating waves will allow applications of controllable artificial atoms in quantum optics and photonics.

A single atom interacting with electromagnetic modes of free space is a fundamental example of an open quantum system

(Fig. 1A) (*1*). The interaction between the atom (or molecule, quantum dot, etc.) and a resonant electromagnetic field is particularly important

for quantum electronics and quantum information processing. In three-dimensional (3D) space, however, although perfect coupling (with 100% extinction of transmitted power) is theoretically feasible (*2*), experimentally achieved extinction has not exceeded 12% (*3–7*) because of spatial mode mismatch between incident and scattered waves. This problem can be avoided by an efficient coupling of the atom to the continuum of electromagnetic modes confined in a 1D transmission line (Fig. 1B), as proposed in (*8, 9*). Here, we

¹NEC Nano Electronics Research Laboratories, Tsukuba, Ibaraki 305-8501, Japan. ²RIKEN Advanced Science Institute, Tsukuba, Ibaraki 305-8501, Japan. ³Department of Physics, Loughborough University, Loughborough, LE11 3TU Leicestershire, UK.

*To whom correspondence should be addressed. E-mail: astf@zb.jp.nec.com

†This author is on leave from Physical-Technical Institute, Tashkent 100012, Uzbekistan.

‡This author is on leave from Lebedev Physical Institute, Moscow 119991, Russia.

demonstrate extinction of 94% on an artificial atom coupled to the open 1D transmission line. The situation with the atom interacting with freely propagating waves is qualitatively different from that of the atom interacting with a single-cavity mode; the latter has been used to demonstrate a series of cavity quantum electrodynamics (QED) phenomena (10–18). Moreover, in open space the atom directly reveals such phenomena known from quantum optics as anomalous dispersion and strongly nonlinear behavior in elastic (Rayleigh) scattering near the atomic resonance (1). Furthermore, spectrum of inelastically scattered radiation is observed and exhibits the resonance fluorescence triplet (the Mollow triplet) (19–23) under a strong drive.

Our artificial atom is a macroscopic superconducting loop, interrupted by Josephson junctions (Fig. 1B) [identical to a flux qubit (24)] and

threaded by a bias flux Φ_b close to a half flux quantum $\Phi_0/2$, and shares a segment with the transmission line (25), which results in a loop-line mutual inductance M mainly due to kinetic inductance of the shared segment (26). The two lowest eigenstates of the atom are naturally expressed via superpositions of two states with persistent current, I_p , flowing clockwise or counterclockwise. In energy eigenbasis, the lowest two levels $|g\rangle$ and $|e\rangle$ are described by the truncated Hamiltonian $H = \hbar\omega_a\sigma_z/2$, where $\omega_a = \sqrt{\omega_0^2 + \varepsilon^2}$ is the atomic transition frequency and σ_i ($i = x, y, z$) are the Pauli matrices. Here, $\hbar\varepsilon = 2I_p\delta\Phi$ ($\delta\Phi \equiv \Phi_b - \Phi_0/2$) is the energy bias controlled by the bias flux, and $\hbar\omega_0$ is the anticrossing energy between the two persistent current states. The excitation energies of the third and higher eigenstates are much larger than $\hbar\omega_0$; therefore, they can be neglected in our analysis.

Fig. 1. Resonance fluorescence: resonant wave scattering on a single atom. (A) Sketch of a natural atom in open space. The atom resonantly absorbs and reemits photons in a solid angle of 4π . (B) False-colored scanning-electron micrograph of an artificial atom coupled to a 1D transmission line. A loop with four Josephson junctions is inductively coupled to the line. The incident wave (blue arrow) is scattered only backward and forward (red arrows) and can be detected in either direction. The transmitted wave is indicated by a magenta arrow. (C) Spectroscopy of the artificial atom. Shown is the power transmission coefficient $|t|^2$ versus flux bias $\delta\Phi$ and incident microwave frequency $\omega/2\pi$. When the incident radiation is in resonance with the atom, a dip of $|t|^2$ reveals a dark line. (Inset) Power transmission coefficient $|t|^2$ at $\delta\Phi = 0$ as a function of incident wave detuning $\delta\omega/2\pi$ from the resonance frequency $\omega/2\pi = 10.204$ GHz. The maximal power extinction of 94% takes place at the resonance ($\delta\Phi = 0$).

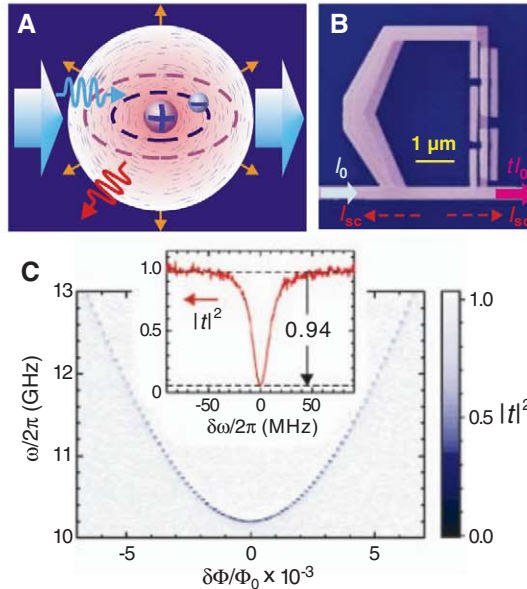
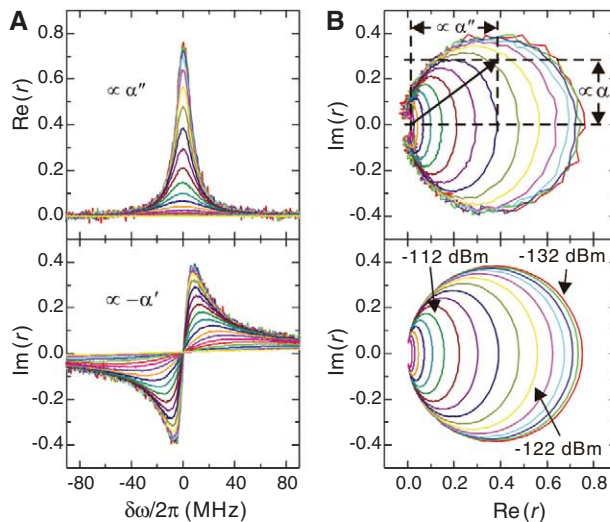


Fig. 2. Elastic scattering of the incident microwave. The reflection coefficient r at $\delta\Phi = 0$ (measured at different powers), being proportional to the atomic polarizability, exhibits “anomalous dispersion.” (A) Real and imaginary parts of r as a function of the detuning frequency $\delta\omega/2\pi$ from the resonance at $\omega_0 = 10.204$ GHz. The driving power W_0 is varied from -132 dBm (largest r) to -84 dBm (smallest r) with an increment of 2 dB. (B) Smith charts of the microwave reflection. (Top) Experimentally obtained r is plotted in the coordinates of $\text{Re}(r)$ and $\text{Im}(r)$ for powers from -132 dBm to -102 dBm with a step of 2 dB. The color coding is the same as in (A). (Bottom) Calculation using Eq. 2 for the same signal powers as in the top panel.



We considered a dipole interaction of the atom with a field of an electromagnetic 1D wave. In the semiclassical approach of quantum optics, the external field of the incident wave $I_0(x, t) = I_0 e^{ikx - i\omega t}$ (where ω is the frequency and k is the wavenumber) induces the atomic polarization. The atom with a characteristic loop size of ~ 10 μm (which is negligibly small as compared with the wavelength $\lambda \sim 1$ cm) placed at $x = 0$ generates waves $I_{sc}(x, t) = I_{sc} e^{ik|x| - i\omega t}$, propagating in both directions (forward and backward). The current oscillating in the loop under the external drive induces an effective magnetic flux ϕ (playing a role of atomic polarization). The net wave $I(x, t) = (I_0 e^{ikx} + I_{sc} e^{ik|x|}) e^{-i\omega t}$ satisfies the 1D wave equation $\partial_{xx} I - v^{-2} \partial_{tt} I = c\delta(x) \partial_{tt} \phi$, where the wave phase velocity is $v = 1/\sqrt{lc}$ (l and c are inductance and capacitance per unit length, respectively), and the dispersion relation is $\omega = vk$.

At the degeneracy point ($\varepsilon = 0$), $\omega_a = \omega_0$, and the dipole interaction of the atom with the electromagnetic wave in the transmission line $H_{\text{int}} = -\phi_p \text{Re}[I_0(0, t)]\sigma_x$ is proportional to the dipole moment matrix element $\phi_p = MI_p$. In the rotating wave approximation, the standard form of the Hamiltonian of a two-level atom interacting with the nearly resonant external field is $H = -(\hbar\delta\omega\sigma_z + \hbar\Omega\sigma_x)/2$. Here, $\delta\omega = \omega - \omega_0$ is the detuning, and $\hbar\Omega = \phi_p I_0$ is the dipole interaction energy. The time-dependent atomic dipole moment can be presented for a negative frequency component as $\langle\phi(t)\rangle = \phi_p \langle\sigma^-\rangle e^{-i\omega t}$, and the boundary condition for the scattered wave generated because of the atomic polarization satisfies the equation $2ik(I_{sc}/2) = -\omega^2 c\phi_p \langle\sigma^-\rangle$, where $\sigma^\pm = (\sigma_x \pm i\sigma_y)/2$. Assuming that the relaxation of the atom is caused solely by the quantum noise of the open line, we obtain the relaxation rate $\Gamma_1 = (\hbar\omega\phi_p^2)/(\hbar^2 Z)$ (where $Z = \sqrt{l/c}$ is the line impedance) (27) and find

$$I_{sc}(x, t) = i \frac{\hbar\Gamma_1}{\phi_p} \langle\sigma^-\rangle e^{ik|x| - i\omega t} \quad (1)$$

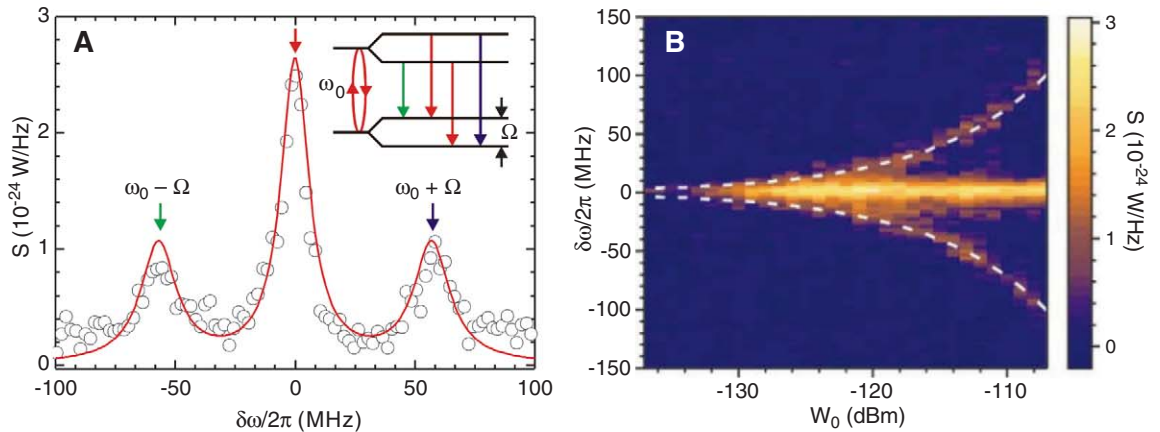
This expression indicates that the atomic dissipation into the line reveals itself even in elastic scattering.

The atom coupled to the open line is described by the density matrix ρ , which satisfies the master equation $\dot{\rho} = -\frac{i}{\hbar} [H, \rho] + \hat{L}[\rho]$. At zero temperature, the simplest form of the Lindblad operator $\hat{L}[\rho] = -\Gamma_1 \sigma_z \rho_e - \Gamma_2 (\sigma^+ \rho_{eg} + \sigma^- \rho_{ge})$ describes energy relaxation (the first term) and the damping of the off-diagonal elements of the density matrix with the dephasing rate $\Gamma_2 = \Gamma_1/2 + \Gamma_\varphi$ (the second term), where Γ_φ is the pure dephasing rates. It is convenient to define reflection and transmission coefficients r and t according to $I_{sc} = -rI_0$ and $I_0 + I_{sc} = tI_0$ and, therefore, $t = 1 - r$. From Eq. 1, we find the stationary solution

$$r = r_0 \frac{1 + i\delta\omega/\Gamma_2}{1 + (\delta\omega/\Gamma_2)^2 + \Omega^2/\Gamma_1\Gamma_2} \quad (2)$$

where the maximal reflection amplitude $r_0 = \eta\Gamma_1/2\Gamma_2$ at $\delta\omega = 0$. Here, η presents dimension-

Fig. 3. Resonance fluorescence triplet: spectrum of inelastically scattered radiation. **(A)** Linear frequency spectral density [$S = 2\pi S(\omega)$] of emission power under a resonant drive with the Rabi frequency of $\Omega/2\pi = 57$ MHz corresponding to the incident microwave power of $W_0 = -112$ dBm or 6.3×10^{-15} W. Experimental data are shown by the open circles. The red solid curve is the emission calculated from Eq. 3 with no fitting parameters. A schematic of the triplet transitions in the dressed-state picture is presented in the inset: The atomic levels split by Ω because of strong driving, and transitions with frequencies $\omega_0 - \Omega$, ω_0 , and $\omega_0 + \Omega$ (marked by colored arrows), give rise to three emission peaks. **(B)** Resonance fluorescence



less coupling efficiency to the line field, including nonradiative relaxation. The maximal possible power extinction ($1 - |r|^2$) can reach 100% when $|r_0| = 1$. It takes place for $\eta = 1$ and $\Gamma_2 = \Gamma_1/2$, that is, in the absence of pure dephasing, $\Gamma_\phi = 0$. In such a case, the wave scattered forward by the atom is canceled out because of destructive interference with the incident wave ($I_{sc} = -I_0$). Although Eq. 2 is obtained for the degeneracy point ($\epsilon = 0$), it remains valid in the general case of $\epsilon \neq 0$ if the dipole interaction energy $\hbar\Omega$ is multiplied by ω_0/ω_a .

The excitation energy of the atom was revealed by means of transmission spectroscopy (Fig. 1C). Owing to the broadband characteristics of the transmission line, we swept the frequency of the incident microwave in a wide range and monitored the transmission. As shown in the inset of Fig. 1C, the resonance is detected as a sharp dip in the power transmission coefficient $|t|^2$. At resonance, the power extinction reaches its maximal value of 94%, which suggests that the system is relatively well isolated from other degrees of freedom in the surrounding solid-state environment and behaves as a nearly isolated atom in open space, coupled only to the electromagnetic fields in the space. The resonance frequency ω_a is traced as a function of the flux bias $\delta\Phi$. By fitting the data, we obtained $\omega_0/2\pi = 10.204$ GHz at $\delta\Phi = 0$ and the persistent current $I_p = 195$ nA.

The elastic response of the artificial atom shows typical anomalous dispersion. Figure 2A represents the reflection coefficient derived from the transmission according to $r = 1 - t$ and obtained at $\delta\Phi = 0$. Similarly to the case of a natural atom, we can define the polarizability $\alpha = \alpha' + i\alpha''$ as $\langle\phi\rangle = \alpha I_0$ and, therefore, $\alpha \propto ir$. In the vicinity of the resonance, $\text{Re}(r) (\propto \alpha')$ is positive and reaches maximum at the resonance, whereas $\text{Im}(r) (\propto -\alpha'')$ changes the sign from positive to negative.

With a weak driving field of $\Omega^2/(\Gamma_1\Gamma_2) \ll 1$ (Fig. 2A, topmost curve), a peak in $\text{Re}(r)$

$\{\text{Re}(r) = \eta r_0[1 + (\delta\omega/\Gamma_2)^2]^{-1/2}\}$ appears. Fitting by using Eq. 2 with $\eta = 1$ gives $\Gamma_1 = 6.9 \times 10^7 \text{ s}^{-1}$ ($\Gamma_1/2\pi = 11$ MHz) and $\Gamma_2 = 4.5 \times 10^7 \text{ s}^{-1}$ ($\Gamma_2/2\pi = 7.2$ MHz). From the expression for Γ_1 , the mutual inductance between the atom and the transmission line is estimated to be $M = 12$ pH. Although our assumption of $\eta = 1$ has not been checked experimentally, it may be reasonable because (i) all the line current should effectively interact with the atom and (ii) the possible relaxation without emission measured for isolated atoms is weak, being typically less than 10^6 s^{-1} (28). In a case of imperfect coupling ($\eta < 1$), the actual Γ_1 could be slightly higher.

The nonlinearity of the atom manifests in the saturation of the atom excitation. With increasing the power of the incident microwave W_0 , $|r|$ monotonically decreases, and in the Smith chart (Fig. 2B) the shape of the trajectory changes from a large circle to a small ellipse. As a single two-level system, the atom is saturated at larger powers and can have large reflectance only for the weak driving case. Again, the nearly perfect agreement between the calculations and the measurements supports our model of a two-level atom coupled to a single 1D mode. Any artificial medium built of such “atoms” (29) will also have a strongly nonlinear susceptibility.

So far, we have investigated elastic Rayleigh scattering in which the incident and the scattered waves have the same frequency. However, the rest of the power $W'_{sc} = W_0(1 - |r|^2 - |r|^2)$ is scattered inelastically and can be observed in the power spectrum. The spectrum was measured at the degeneracy point ($\delta\Phi = 0$) under a resonant drive with the power corresponding to $\Omega/2\pi \approx 57$ MHz (Fig. 3A). It manifests the resonance fluorescence triplet, also known as the Mollow triplet (19–23). In the case of a strong driving field ($\Omega^2 \gg \Gamma_1^2$), the expression for the inelastically scattered power simplifies to $W'_{sc} \approx (\Gamma_1^2/\Omega^2)W_0$, which is independent of the incident power and can be rewritten as $W'_{sc} \approx$

emission spectrum as a function of the driving power. The dashed white lines indicate the calculated position of the side peaks shifted by $\pm\Omega/2\pi$ from the main resonance. The split peak was used for calibration of the field amplitude at the atom.

$\hbar\omega\Gamma_1/2$: The atom is half populated by the strong drive and spontaneously emits with rate Γ_1 . Assuming $\eta = 1$, the spectral density measured in one of the two directions is expected to be

$$S(\omega) \approx \frac{1}{2\pi} \frac{\hbar\omega\Gamma_1}{8} \left(\frac{\gamma_s}{(\delta\omega + \Omega)^2 + \gamma_s^2} + \frac{2\gamma_c}{\delta\omega^2 + \gamma_c^2} + \frac{\gamma_s}{(\delta\omega - \Omega)^2 + \gamma_s^2} \right) \quad (3)$$

where half-width of the central and side peaks are $\gamma_c = \Gamma_2$ and $\gamma_s = (\Gamma_1 + \Gamma_2)/2$, respectively. The red curve in Fig. 3A is drawn by using Eq. 3 without any fitting parameters. The good agreement with the theory indicates the high collection efficiency of the emitted photons, which is due to the 1D confinement of the mode. The shift of the side peaks, $\pm\Omega$, from the main resonance depends on the driving power. The intensity plot in Fig. 3B shows how the resonance fluorescence emission depends on the driving power. The dashed white lines mark the calculated position of the side peaks as a function of the driving power, showing good agreement with the experiment.

The demonstrated resonance wave scattering from a macroscopic “artificial atom” in an open transmission line indicates that such superconducting quantum devices can be used as building blocks for controllable, quantum-coherent macroscopic artificial structures, in which a plethora of effects can be realized from quantum optics of atomic systems.

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Materials and Methods

Fig. S1

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Spin-Dependent Quantum Interference Within a Single Magnetic Nanostructure

H. Oka, P. A. Ignatiev, S. Wedekind, G. Rodary,* L. Niebergall, V. S. Stepanyuk,† D. Sander,† J. Kirschner

Quantum interference is a coherent quantum phenomenon that takes place in confined geometries. Using spin-polarized scanning tunneling microscopy, we found that quantum interference of electrons causes spatial modulation of spin polarization within a single magnetic nanostructure. We observed changes in both the sign and magnitude of the spin polarization on a subnanometer scale. A comparison of our experimental results with *ab initio* calculations shows that at a given energy, the modulation of the spin polarization can be ascribed to the difference between the spatially modulated local density of states of the majority spin and the nonmodulated minority spin contribution.

When electrons are confined to nanostructures, their dual wave-particle nature comes into full view in experiment. In particular, spatially modulated variations of the electronic local density of states (LDOS) have been observed by scanning tunneling microscopy (STM) (1, 2). These modulation patterns reflect quantum interference between electron waves that are scattered off the boundaries of a nanostructure, forming a standing wave.

In addition to charge, electrons also carry a spin. When confined to magnetic nanostructures, an imbalance between electrons of opposite spin orientations leads to spin-polarized electron waves, giving rise to spin-dependent quantum interference. Recent theoretical studies predicted that local spin polarization on nonmagnetic surfaces as well as on magnetic nanostructures can be spatially modulated (3–5).

We observed spin-dependent quantum interference by means of spin-polarized STM (SP-STM)

(6), a technique sensitive to surface magnetization. The nanostructures we study are triangular Co islands located on a nonmagnetic substrate, the (111) surface of copper (4, 7–10). A previous study, which investigated a system similar to ours but under different conditions, indicated the dependence of electron confinement on the spin character of electronic states (9).

The SP-STM measurements were performed at a temperature of 8 K. To detect magnetic contrast, we used W tips covered with magnetic materials (Cr-Co-W tips) and external magnetic fields up to $B = 4$ T. To explore the spatial distribution of the spin polarization, we measure the spatially resolved map of the differential tunneling conductance (dI/dV) asymmetry, $A_{dI/dV}$, defined by

$$A_{dI/dV} \equiv \frac{dI/dV_{AP} - dI/dV_P}{dI/dV_{AP} + dI/dV_P} \quad (1)$$

where dI/dV_{AP} and dI/dV_P are the dI/dV signals measured with the tip and sample magnetization in antiparallel (AP) and parallel (P) configurations, respectively. The dI/dV signal depends on the relative orientation of the tip and sample magnetizations, which is described in the gen-

eralized Tersoff-Hamann model (11, 12). Using that model, we can link the dI/dV asymmetry $A_{dI/dV}$ to the spin polarization of the tip, P_T , and of the sample at the tip apex position, P_S (10, 13):

$$A_{dI/dV} = -P_T P_S \quad (2)$$

A constant-current STM image (Fig. 1) shows a typical triangular Co island on Cu(111) (14, 15). To link the dI/dV asymmetry with the spin polarization of the sample, we characterize the magnetization orientation of the Co island with respect to the magnetic tunneling tip. A dI/dV hysteresis loop, in which we plot the dI/dV signal as a function of the magnetic field at a fixed energy, allows us to identify the magnetic configurations (fig. S1) (16–18).

To extract the $A_{dI/dV}$ maps of the Co island, we recorded two dI/dV images on the exact same island with AP (Fig. 2A) and P (Fig. 2B) magnetization configurations, at a bias voltage of $V = +0.03$ V. Note that it is essential to map the dI/dV signal with P and AP configurations on exactly the same island because the electronic structures of Co islands on Cu(111) strongly depend on the size, stacking, and shape of the island, as well as position within the island (8, 9, 19).

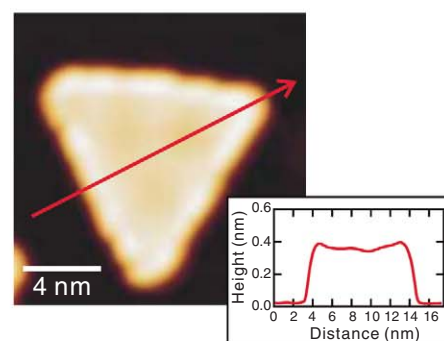


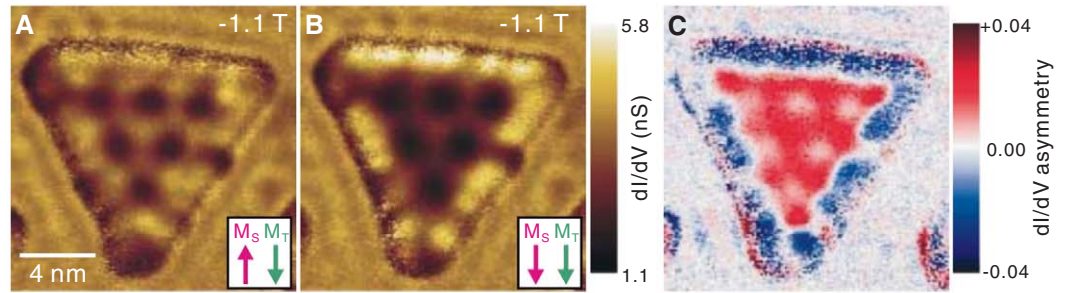
Fig. 1. Constant-current STM image of a triangular Co island on Cu(111); $V_s = -0.1$ V, $I = 1.0$ nA, where V_s is the sample bias voltage with respect to the tip and I is the tunneling current. The inset shows a line profile along the red arrow in the STM image. The Co island is two atomic layers (~ 0.4 nm) in height and has a base length of 12 nm.

Max-Planck-Institut für Mikrostrukturphysik, Weinberg 2, D-06120 Halle/Saale, Germany.

*Present address: Laboratoire de Photonique et de Nanostructures, CNRS UPR20, Route de Nozay, 91460 Marcoussis, France.

†To whom correspondence should be addressed. E-mail: stepanyuk@mpi-halle.de (V.S.S.); sander@mpi-halle.de (D.S.)

Fig. 2. (A and B) Two dI/dV images of the Co island in Fig. 1, the basis for the dI/dV asymmetry map in (C). Both images were recorded at $B = -1.1$ T, but with different magnetization configurations between the magnetic tunneling tip and the Co island: antiparallel (A) and parallel (B). The insets represent the antiparallel (AP) and parallel (P) configurations. $V = +0.03$ V, $V_{\text{stab}} = +0.5$ V, and $I = 1.0$ nA, where V is the bias voltage at which the dI/dV signal is recorded and V_{stab} is the bias voltage to stabilize the tip before the feedback loop is opened (10). (C) dI/dV asymmetry map calculated using Eq. 1 from the images in (A) and (B).



We used Eq. 1 to calculate the dI/dV asymmetry map (Fig. 2C) from the two dI/dV images. The dI/dV asymmetry near the Fermi level at $V = +0.03$ V is strongly position-dependent within the Co island. At the edge of the island it is negative, whereas the inner part of the island shows largely positive values. This result can be explained by the existence of a rim state, which is localized spatially around the edges of the Co island and energetically around the Fermi level (9). The rim state originates from a minority d state (9), whereas the inner part of the island mainly has the opposite spin character around the Fermi level and is of majority s-p surface state (4, 7). The magnetization of the tip did not change direction, and the applied bias voltage was fixed at $V = +0.03$ V during the two measurements of the dI/dV image. Thus, we can assume that the spin polarization of the tip, P_T , is constant and the dI/dV asymmetry is proportional to the spin polarization of the sample—that is, $A_{dI/dV} \propto P_S$. Consequently, we conclude that the inner part of the Co island exhibits a positive dI/dV asymmetry because the electronic state with the majority spin character is dominant, and that the edge of the Co island exhibits a negative dI/dV asymmetry because the electronic state with the minority spin character is dominant. To interpret the spatial modulation in the dI/dV asymmetry (Fig. 2C), which is not due to the atomic structure of the Co island, we focus our discussion on the inner part of the island and analyze the system theoretically.

Our analysis uses density functional theory implemented in the multiple-scattering Korringa-Kohn-Rostoker Green's function method (10, 20) to calculate spatially resolved maps of the LDOS above a triangular two-atomic-layer Co island on Cu(111) for the majority (Fig. 3A) and minority (Fig. 3B) spins at the Fermi level. The LDOSs for the majority spins are spatially modulated inside the triangular Co island. We ascribe this to a standing wave induced by the electronic confinement of the free electron-like surface state. Minority spins have a constant LDOS, consistent with the understanding that they are mainly due to energetically and spatially localized d states, which are hardly influenced by the electronic confinement (4). We extract the spatial distribution of the spin polarization above

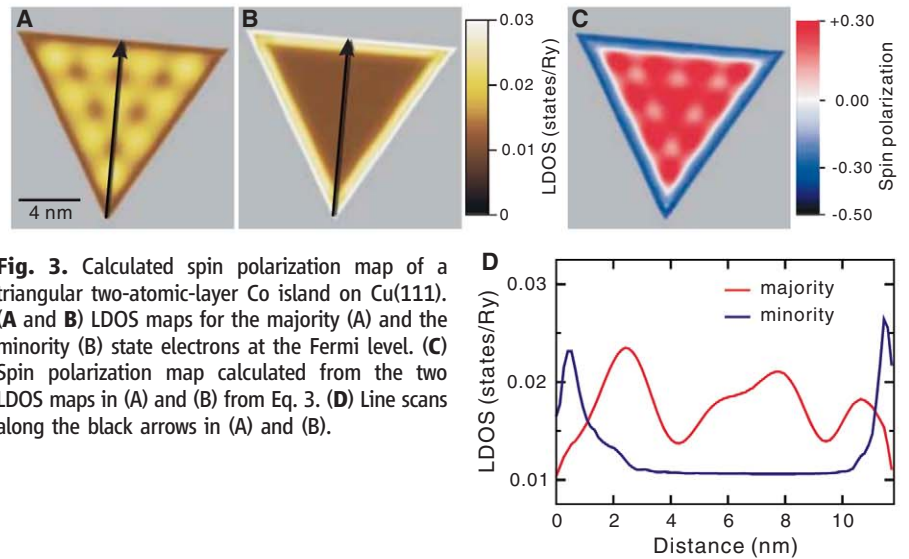


Fig. 3. Calculated spin polarization map of a triangular two-atomic-layer Co island on Cu(111). (A and B) LDOS maps for the majority (A) and the minority (B) state electrons at the Fermi level. (C) Spin polarization map calculated from the two LDOS maps in (A) and (B) from Eq. 3. (D) Line scans along the black arrows in (A) and (B).

the Co island, P_{Co} (Fig. 3C), from the two calculated LDOS maps as

$$P_{\text{Co}} = \frac{n_{\uparrow} - n_{\downarrow}}{n_{\uparrow} + n_{\downarrow}} \quad (3)$$

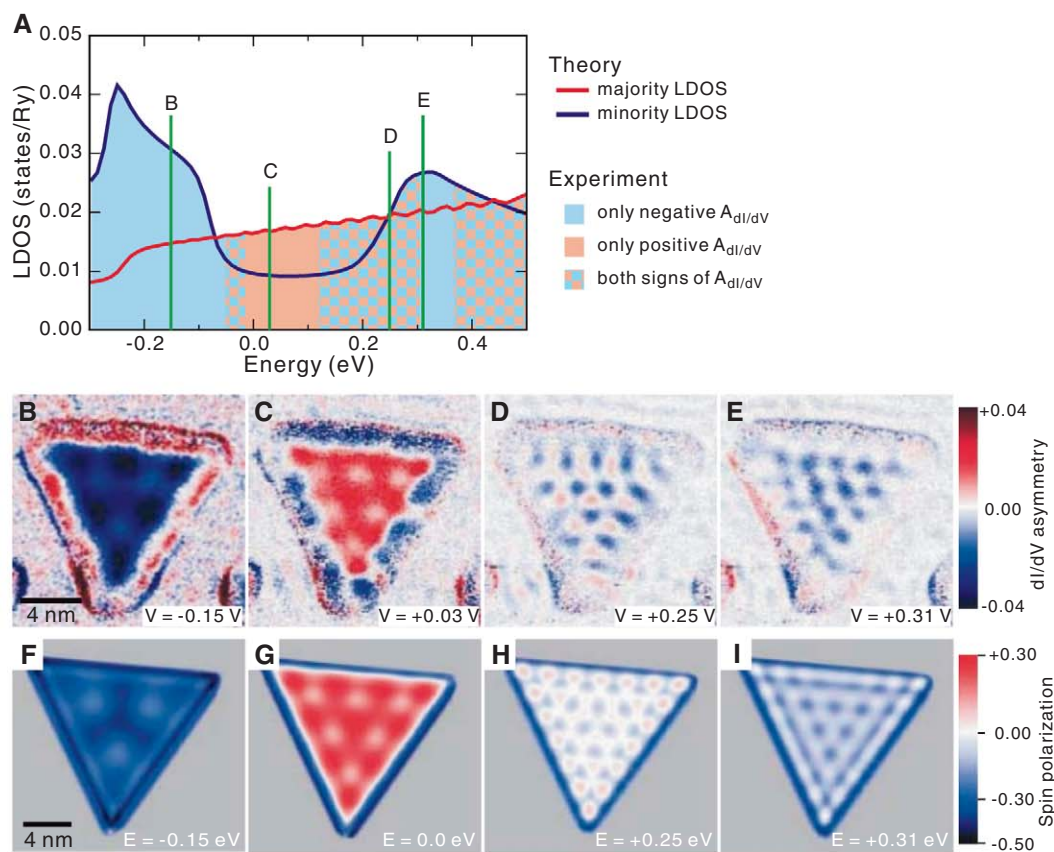
where $n_{\uparrow}$ and $n_{\downarrow}$ are the LDOS in the vacuum region above the Co island for the majority and minority spins, respectively (13). To identify the origin of the largely positive spin polarization observed in Fig. 3C, we present two line profiles (Fig. 3D) of the LDOS maps of Fig. 3, A and B. At the Fermi level, the spatially modulated LDOS of the majority spin is larger everywhere in the inner part of the island than the spatially flat LDOS of the minority spin. This leads to a positive spin polarization, $P_{\text{Co}} > 0$ in Eq. 3, in this region.

Comparing Figs. 2C and 3C, we find good agreement between the measured dI/dV asymmetry map and the calculated spin polarization map within the inner part of the Co island. Therefore, the dI/dV asymmetry map of the Co island can be interpreted as follows: (i) The dI/dV asymmetry map qualitatively shows a spatial distribution of the spin polarization on the Co island at a certain energy (figs. S2 and S3) (21). (ii) The modulation pattern observed in the dI/dV asymmetry map mainly originates from that in the

LDOS of the majority spin, which is ascribed to the electron quantum confinement of the free electron-like s-p surface state. Thus, the spin polarization within the Co island is spatially modulated because of spin-dependent quantum interference.

Next, we examine the energy dependence of the dI/dV asymmetry maps of the same Co island to ensure the interpretation (ii) described above. The modulation pattern in the dI/dV asymmetry maps should change with electron energy. The dI/dV asymmetry maps (Fig. 4, B to E) show clear spatial modulations, similar to those in the corresponding dI/dV images (fig. S4), with modulation patterns strongly dependent on energy. This is easily understood by considering the origin of the modulation pattern, which is electron confinement of the free electron-like s-p surface state. The s-p surface state starts around -0.2 eV below the Fermi level and exhibits a parabolic dispersion with a positive effective mass (7, 9). With increasing energy, the parallel wave vector ($k_{\parallel}$) of the s-p surface state increases; that is, the wavelength (λ) of the standing waves becomes shorter. The modulation pattern observed in the dI/dV asymmetry maps shows the same trend as the standing wave pattern in the dI/dV images. This result corroborates the interpretation (ii) of the dI/dV

Fig. 4. Energy dependences of the measured dI/dV asymmetry maps and calculated spin polarization maps of the Co islands. **(A)** Calculated spin-resolved LDOS of a two-atomic-layer Co film on Cu(111). **(B to E)** Experimental dI/dV asymmetry maps measured on the Co island of Fig. 1. The dI/dV asymmetry maps are calculated from two dI/dV images measured at AP and P states from Eq. 1. Measurement conditions of dI/dV images: $B = -1.1$ T, $V_{\text{stab}} = +0.5$ V, $I = 1.0$ nA. **(F to I)** Calculated spin polarization maps of the triangular Co island. The spatial dependence of the spin polarization as defined by Eq. 3 is shown by the maps, which are calculated from two LDOS maps for the majority and the minority states. Vertical green lines in (A) correspond to the energy positions where the dI/dV asymmetry maps are obtained. A color map in (A) indicates the energy area where experimental results for the inner part of the Co island show only positive (blue), only negative (red), or both signs (lattice pattern with blue and red) of the dI/dV asymmetry in the dI/dV asymmetry maps.



asymmetry maps. Unexpectedly, we also find in Fig. 4, B to E, that the sign of the dI/dV asymmetry changes with respect to energy, whereas the map at $V = +0.25$ V (Fig. 4D) reveals oscillatory change of the sign as a function of position.

To explore the physics behind these surprising results, we plotted a calculated spin-resolved LDOS above a bilayer Co film on Cu(111) as a function of energy (Fig. 4A). Here we focus on the energy range where the free electron-like s-p surface state arises. The LDOS for the majority spin monotonically increases from $E = -0.25$ eV with increasing energy, which is ascribed to the free electron-like s-p surface state. In contrast, the LDOS for the minority spin shows two energetically localized d states around $E = -0.25$ eV and $+0.30$ eV, and it is featureless in the interval between them. The spin-resolved LDOS plots reveal that the dominant spin character strongly depends on energy. We can never conclude that the majority spin character is dominant, even in the energy range where the standing wave pattern, which results from the majority s-p surface state, is clearly observed. We must precisely take into account the dominant spin character at a given energy as revealed by the calculations to evaluate the spatial distribution of the dI/dV asymmetry within the Co island.

At $V = -0.15$ V (Fig. 4B), the inner part of the Co island has a modulated negative value of the dI/dV asymmetry. The spin-resolved LDOS plot (Fig. 4A) shows that the minority spin state

is dominant at the corresponding energy, $E = -0.15$ eV, as the localized minority d state exists. Thus, the spatially flat LDOS of the minority spin exceeds the spatially modulated LDOS of the majority spin inside the Co island. This leads to the negative spin polarization inside the island, $P_{\text{Co}} < 0$, which comes from a negative numerator in Eq. 3 (i.e., $n_{\uparrow} - n_{\downarrow} < 0$), in agreement with the calculated spin polarization map (Fig. 4F). We note that the periodicity of the standing waves in the energy range considered, ≥ 1.5 nm, is much larger than the distance between neighboring Co atoms, ~ 0.2 nm.

At $E = +0.25$ eV, where the spin-resolved LDOS plots of the majority and the minority spins cross, we expect that the net spin polarization should be zero within the Co island. The spatial distribution of the LDOS of the majority spin is, however, not flat but modulated. The LDOS of the majority spins is larger than that of the minority at a convexity of the standing wave, and the resulting spin polarization is positive. Correspondingly, the LDOS of the majority spins is smaller than that of the minority at a concavity of the standing wave, and the resulting spin polarization is negative. Where the LDOS of the majority spins is comparable to that of the minority at a node of the standing wave, the resulting spin polarization is zero. Therefore, the spin polarization map shows a spatial modulation and the sign of the spin polarization oscillates as a function of position, depending on the magnitude

of the modulated LDOS of the majority spin (Fig. 4H).

We add a color map to Fig. 4A to clarify the energy window where the experimental dI/dV asymmetry map exhibits only positive, only negative, or both signs of the dI/dV asymmetry within the inner part of the Co island. We find that the dominant spin character at a given energy governs the sign of the dI/dV asymmetry and its spatial distribution.

The finding that not only the magnitude but also the sign of the spin polarization is spatially and energetically modulated within a single magnetic nanostructure implies that we can easily change or control local spin polarization on a magnetic nanostructure by changing the energy at a given position, or the position at a given energy.

Our method for extracting the dI/dV asymmetry map, which requires that the relative magnetization directions of sample and magnetic tip are experimentally determined by in-field SP-STM, allows us to qualitatively visualize the spin polarization of a single nanostructure on an angstrom scale. Recent ab initio calculations predicted that the spin polarization of surface-state electrons on Cu(111) caused by magnetic adatoms can be enhanced within a Cu corral, and the quantum confinement of surface electrons within corrals or islands can be exploited to tailor the exchange interaction between magnetic adatoms (3, 22). When combined with the capability of the STM to manipulate adatoms and assemble engineered nanostructures (23, 24), our method offers a way

to explore and directly image the exchange interaction and the role of conduction electrons mediating the interaction, such as the Ruderman-Kittel-Kasuya-Yosida interaction.

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Multiple Functional Groups of Varying Ratios in Metal-Organic Frameworks

Hexiang Deng, Christian J. Doonan, Hiroyasu Furukawa, Ricardo B. Ferreira, John Towne, Carolyn B. Knobler, Bo Wang, Omar M. Yaghi*

We show that metal-organic frameworks (MOFs) can incorporate a large number of different functionalities on linking groups in a way that mixes the linker, rather than forming separate domains. We made complex MOFs from 1,4-benzenedicarboxylate (denoted by “A” in this work) and its derivatives -NH₂, -Br, -(Cl)₂, -NO₂, -(CH₃)₂, -C₄H₄, -(OC₃H₅)₂, and -(OC₇H₇)₂ (denoted by “B” to “I,” respectively) to synthesize 18 multivariate (MTV) MOF-5 type structures that contain up to eight distinct functionalities in one phase. The backbone (zinc oxide and phenylene units) of these structures is ordered, but the distribution of functional groups is disordered. The complex arrangements of several functional groups within the pores can lead to properties that are not simply linear sums of those of the pure components. For example, a member of this series, MTV-MOF-5-EHI, exhibits up to 400% better selectivity for carbon dioxide over carbon monoxide compared with its best same-link counterparts.

Crystalline extended structures are usually considered “simple” because they are constructed from a small number of distinct building units. Attempting to increase the number of such units in solids generally leads to either mixed phases, rather than a single phase of mixed units, or amorphous materials. In block copolymers, a minor modification to the side chains alters the entropy of the system and results in major (and often undesirable) changes in the structure of the polymer (1). Here, we show that by combining the inherent rigidity of metal-organic frameworks (MOFs) and the functional flexibility of polymers, one can overcome these challenges and create a large number of single-phase materials, each of which has multivariate (MTV) functionalities.

California Nanosystems Institute, University of California—Los Angeles (UCLA)—Department of Energy (DOE) Institute of Genomics and Proteomics, Department of Chemistry and Biochemistry, UCLA, 607 Charles E. Young Drive East, Los Angeles, CA 90095, USA.

*To whom correspondence should be addressed. E-mail: yaghi@chem.ucla.edu

Our strategy to making MTV-MOFs is to assemble their structures from links with different functional groups whose orientation, number, relative position, and ratio along the backbone (metal-oxide and phenylene units) can be controlled by virtue of the unchanged length of the link and its unaltered connectivity (Scheme 1). Such a construct can be viewed as having a primary structure composed of the simple repeating pattern of metal-oxide joints and organic links and a “complex” secondary structure formed by multivariate arrangements of many functional groups that are covalently bound to the links. In this way, each of the pores within the MOF would have an array of functionalities pointing into its center. Accordingly, the sequence of such functionalities and the frequency with which certain ones appear in the sequence will endow the pores with a new level of complexity that far exceeds any held by that of the original same-link MOFs—an aspect that may allow fine-tuning of the pore environment with favorable implications on properties.

Our past work (as well as the work of others) has shown that MOFs with two mixed links can be prepared, whereas a recent report showed that four different functionalities can be introduced into one structure by post-synthesis modification (2–7). These approaches are either confined to only two links or severely limited by having complete reactions at the links; multiple variations in link ratios and functionalities in these systems were not demonstrated. The present report describes a general method for producing crystalline MOF materials that combine sets of two to eight links of different functional groups; each set is incorporated into a single structure where the ratio of links is controlled, and the material can be produced with bulk purity. [Hereafter, A, 1,4-benzenedicarboxylate; B, -NH₂; C, -Br; D, -(Cl)₂; E, -NO₂; F, -(CH₃)₂; G, -C₄H₄; H, -(OC₃H₅)₂; and I, -(OC₇H₇)₂.] Specifically, we targeted the cubic MOF-5 structure (8) and combined the acid form of 1,4-benzenedicarboxylate (BDC), NH₂-BDC, Br-BDC, (Cl)₂-BDC, NO₂-BDC, (CH₃)₂-BDC, C₄H₄-BDC, (OC₃H₅)₂-BDC, and (OC₇H₇)₂-BDC links (Scheme 2, A to I, respectively) to form the corresponding sets of 18 MTV-MOFs, each having two or more different functionalities [two: MTV-MOF-5-AB, -AC, -AD, -AE, -AF, -AG, -AH, -AI, and -EI; three: MTV-MOF-5-ABC, -AHI, and -EHI; four: MTV-MOF-5-ABCD and -ACEF; five: MTV-MOF-5-ABCHI; six: MTV-MOF-5-ABCGHI; seven: MTV-MOF-5-ABCEGHI; eight: MTV-MOF-5-ABCEFGHI (Scheme 2)]. We describe their isolation as single phases, the structure of their MOF backbone, and their porosity, and show that this multivariate link synthetic strategy is useful for introducing functionalities [such as NO₂-BDC and (Cl)₂-BDC] into the MOF-5 type structure (MTV-MOF-5-AD and -AE) that do not form this structure when used alone. We also report our initial findings that members of this series (MTV-MOF-5-AHI and -EHI) show that the “whole is better than the sum of its parts,” as evidenced by the fourfold enhancement of gas adsorption and separation properties of the multivariate

link MOFs compared with their simple same-link analogs.

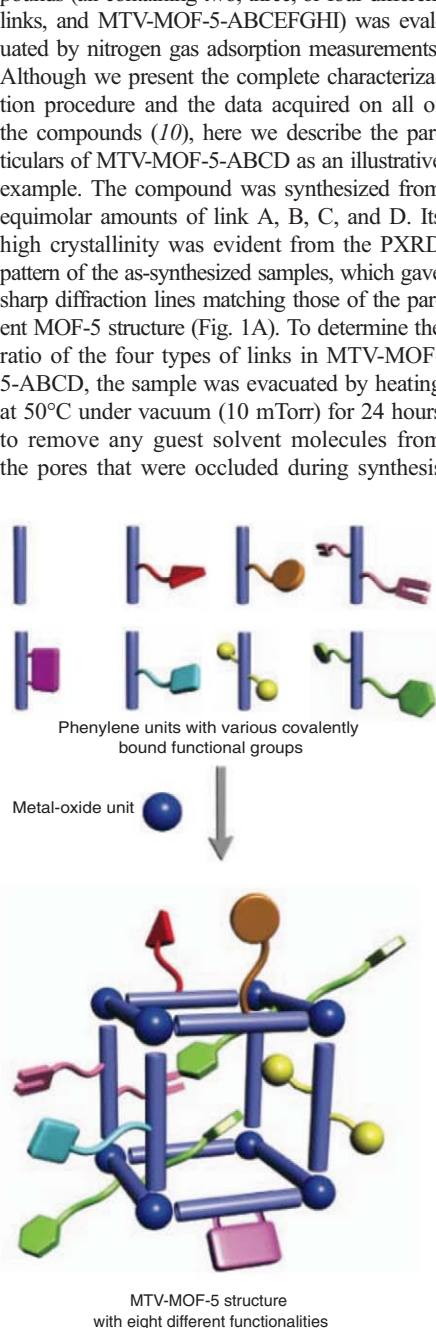
Crystals of MTV-MOFs were obtained by adding $\text{Zn}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ to a *N,N*-dimethylformamide solution mixture of the acid form of the selected organic links under conditions previously used in the synthesis of MOF-5 (8, 9). All of the compounds were characterized by powder x-ray diffraction (PXRD), ^{13}C cross-polarization/magic angle spinning nuclear magnetic resonance (^{13}C CP/MAS NMR), ^1H NMR on acid-digested solutions of their crystals, and thermogravimetric analysis (TGA) to assess their crystallinity, link composition, link ratio, and thermal stability, respectively. The porosity of a subset of these compounds (all containing two, three, or four different links, and MTV-MOF-5-ABCEFGHI) was evaluated by nitrogen gas adsorption measurements. Although we present the complete characterization procedure and the data acquired on all of the compounds (10), here we describe the particulars of MTV-MOF-5-ABCD as an illustrative example. The compound was synthesized from equimolar amounts of link A, B, C, and D. Its high crystallinity was evident from the PXRD pattern of the as-synthesized samples, which gave sharp diffraction lines matching those of the parent MOF-5 structure (Fig. 1A). To determine the ratio of the four types of links in MTV-MOF-5-ABCD, the sample was evacuated by heating at 50°C under vacuum (10 mTorr) for 24 hours to remove any guest solvent molecules from the pores that were occluded during synthesis

(10). TGA performed on this sample showed no weight loss up to 400°C , confirming that all guest molecules were removed from the pores and that the evacuated framework is thermally stable.

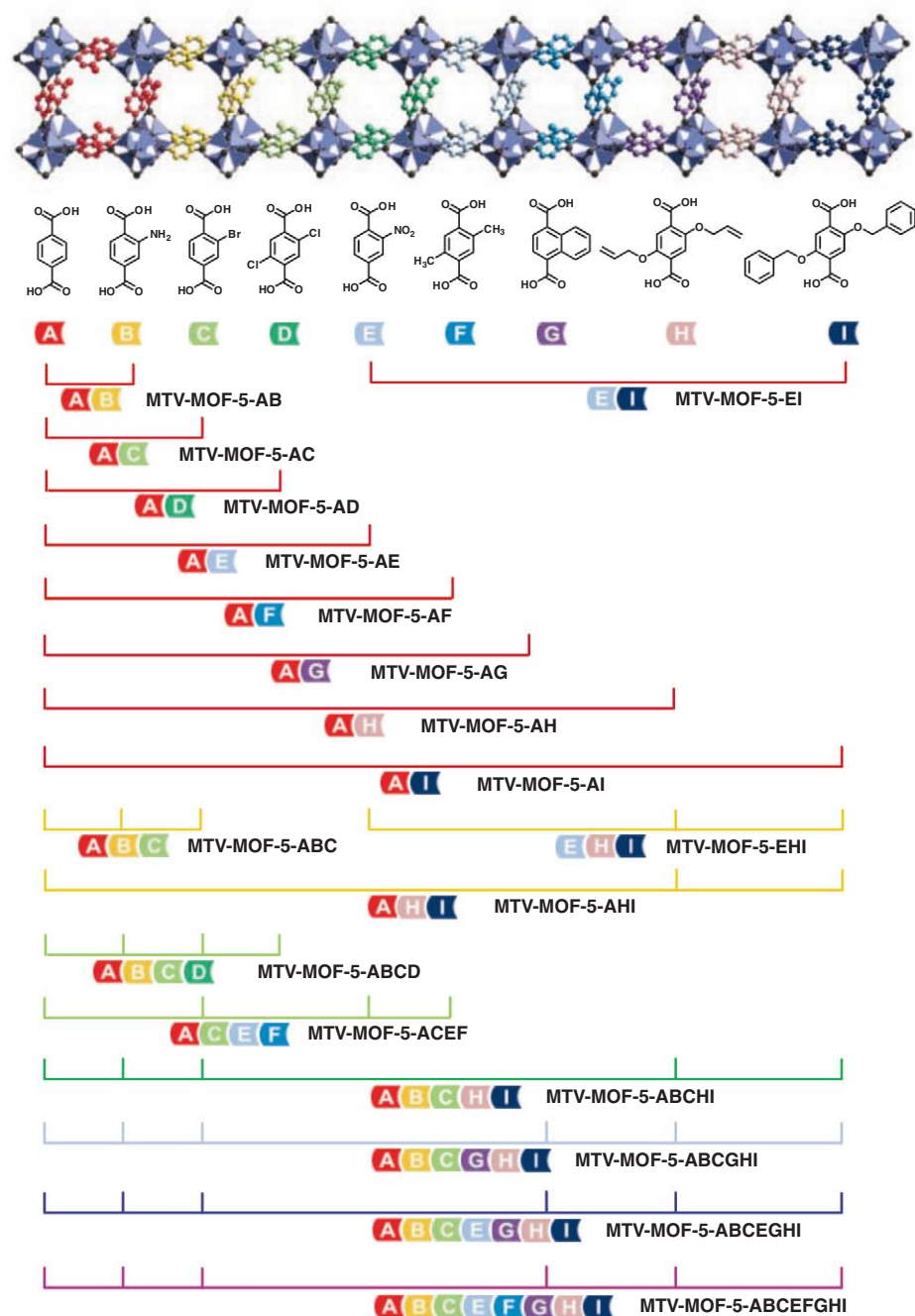
^{13}C CP/MAS NMR spectra of evacuated samples of MTV-MOF-5-ABCD showed resonances at 150.3, 127.0, 133.7, and 136.3 parts per million (ppm), characteristic of the unique carbon atoms of NH_2 -BDC, Br-BDC, $(\text{Cl})_2$ -BDC, and BDC links, respectively (Fig. 1B). These spectra indicate the presence of these units in the MOF backbone. Additionally, the same experiment was performed on a mixture of the constituent free links of MTV-MOF-5-

ABCD, where a distinct shift of 2 ppm was observed between the carbonyl carbons of the free links and those of the links that are incorporated into the framework, thus confirming that no unbound organic link is present within the MOF crystals. Similar analyses on all the remaining MTV-MOFs led to the same conclusion (10).

The precise link ratio was obtained from the ^1H NMR spectra of a DCI-digested solution of the MTV-MOF-5-ABCD solid (Table 1, link composition). Resonances with the predicted coupling patterns were observed in the expected regions for each of the unique protons of the links (Fig. 1C) (10). By integrating resonance peak intensities, we find that the

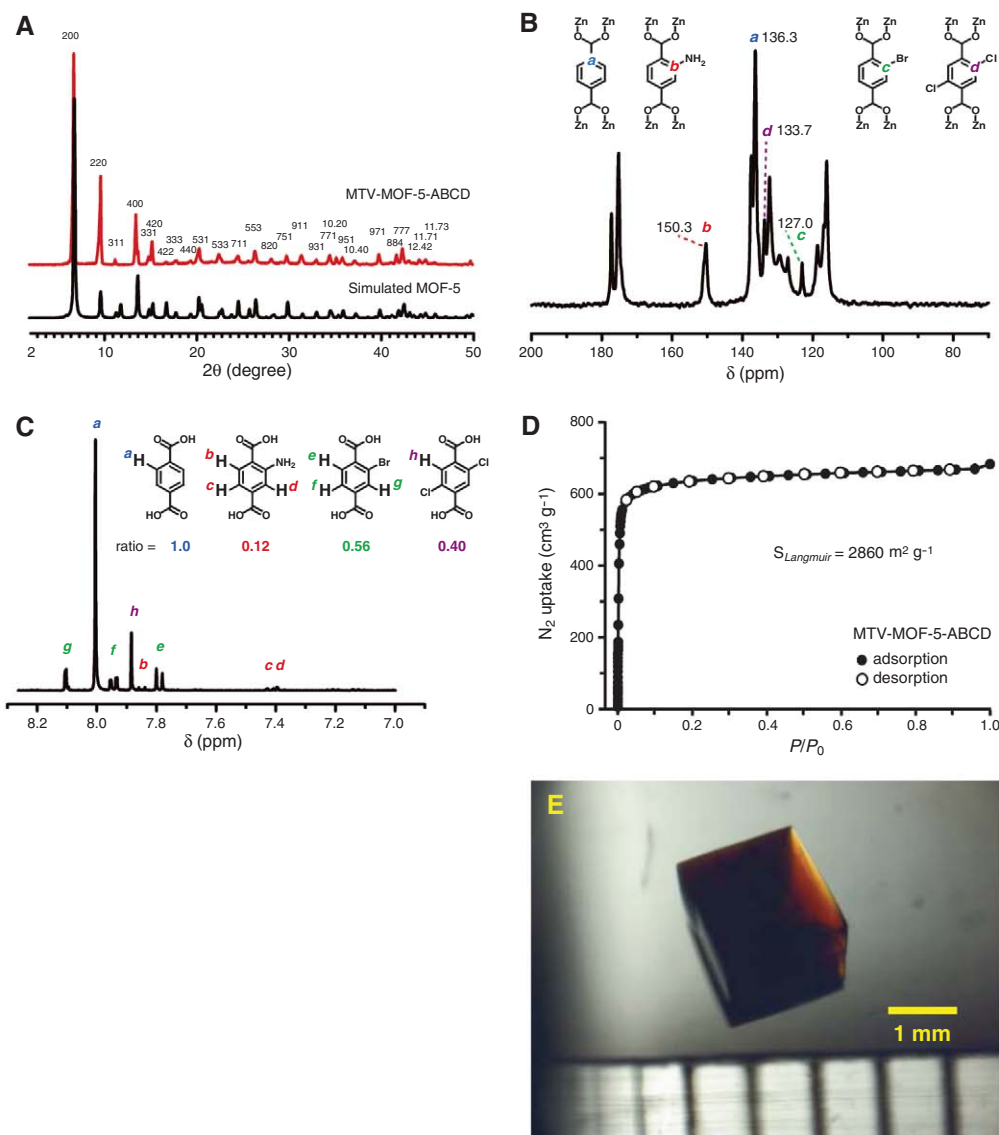


Scheme 1.



Scheme 2.

Fig. 1. Typical analysis performed on MTV-MOFs shown here for samples of MTV-MOF-5-ABCD. **(A)** X-ray diffraction patterns of the crystalline powder compared with the simulated one for MOF-5. **(B)** ^{13}C CP/MAS NMR spectrum showing unique resonance for each link. **(C)** Solution ^1H NMR spectrum used to determine the ratio of links (A: B: C: D = $1.0 \pm 0.12 \pm 0.03$: 0.56 ± 0.04 : 0.40 ± 0.03). **(D)** N_2 adsorption isotherm at 77 K with adsorption and desorption points represented by closed circles and open circles, respectively. S_{Langmuir} , Langmuir surface area; P/P_0 , relative pressure. **(E)** A large crystal from which segments were analyzed for the ratio of links and found to be identical throughout.



links are present in the MOF in the proportion 1.00: 0.12: 0.56: 0.40 (A: B: C: D, respectively), versus an equimolar starting pool. Solution ^1H NMR experiments on four different crystals randomly selected from the MTV-MOF-5-ABCD bulk sample and showed that the ratios stay nearly identical. The same experiment was also performed on MTV-MOF-5-AB and -ABCEFGHI, again confirming the bulk homogeneity of the MTV-MOF series (table S1). Furthermore, the porosity and architectural stability of the original MOF-5 structure are preserved in the MTV-MOF compounds (10), as illustrated by the type I nitrogen adsorption isotherm (shown in Fig. 1D for MTV-MOF-5-ABCD) and its high surface area ($2860 \text{ m}^2 \text{ g}^{-1}$). In addition, by synthesizing MTV-MOF-ABCD, we used a variety of link molar ratios to demonstrate that, in a given MTV-MOF, the link ratio can be controlled by modifying the reaction stoichiometry (Table 1, control of link ratio). In essence, this type of control in link ratios translates into control of the population and diversity of functional groups pointing into

the pores without altering the underlying connectivity of the primary structure, as evidenced by their preserved PXRD patterns (fig. S40).

As expected, x-ray crystallographic studies performed on single crystals of MTV-MOF-5-AC and -ACEF revealed an ordered cubic MOF-5 structure composed of rigid phenylene units joined by $\text{Zn}_4\text{O}(\text{CO}_2)_6$ vertices. The nonhydrogen atoms of the functional groups on the phenylene units in these materials are all present at very low occupancy. Each functional group is required by symmetry to be disordered over two (dimethyl groups of link F) or four (Br group of link C or nitro group of link E) positions because of an equal probability of their location on the four carbon atoms of the phenylene ring. Parameters for Br in MTV-MOF-5-AC can be refined, despite its low occupancy and the low contribution to the intensity of the data. In MTV-MOF-5-ACEF, the occupancies of functional group atoms are also quite low; however, because there is overlap of the positions of Br (link C), N (link E), and C (link F) atoms, a difference peak could be

located. Given that phenylene unit atoms are present in all MTV-MOFs, all of these parameters were successfully refined for the backbone nonhydrogen atoms. This result indicates that the structures of MTV-MOFs are not solid solutions. Thus, they represent a unique crystalline material in which a variable distribution of functional groups is covalently linked to an ordered framework.

The x-ray analysis cannot address whether the crystals are composed of macroscopic domains of functionalities or whether they have distinct sequences of functional units repeated throughout the framework backbone. To distinguish these two possibilities, we prepared large single crystals of MTV-MOF-5-AB, -ABCD (Fig. 1E), and -ABCEFGHI of dimensions of 4.0 mm by 4.0 mm by 2.0 mm, 2.0 mm by 2.0 mm by 2.0 mm, and 2.0 mm by 2.0 mm by 1.0 mm, respectively (fig. S33). In each case, the structure of each single crystal was confirmed by its x-ray diffraction pattern (figs. S34 to S36). Each crystal was dissected into three equal segments,

Table 1. Ratio of links determined for MTV-MOF crystals and their respective reaction stoichiometry (shown in parentheses). In each case, the ratios were normalized to a value of one for link A. NA, not applicable.

Compound	A, (A)	B, (B)	C, (C)	D, (D)	E, (E)	F, (F)	G, (G)	H, (H)	I, (I)
<i>Link composition</i>									
MTV-MOF-5-AB	1.0, (1)	0.57, (1)	NA	NA	NA	NA	NA	NA	NA
MTV-MOF-5-AC	1.0, (1)	NA	0.61, (1)	NA	NA	NA	NA	NA	NA
MTV-MOF-5-AD	1.0, (1)	NA	NA	0.63, (1)	NA	NA	NA	NA	NA
MTV-MOF-5-AE	1.0, (1)	NA	NA	NA	0.40, (1)	NA	NA	NA	NA
MTV-MOF-5-AF	1.0, (1)	NA	NA	NA	NA	1.24, (1)	NA	NA	NA
MTV-MOF-5-AG	1.0, (1)	NA	NA	NA	NA	NA	0.52, (1)	NA	NA
MTV-MOF-5-AH	1.0, (1)	NA	NA	NA	NA	NA	NA	0.46, (1)	NA
MTV-MOF-5-AI	1.0, (1)	NA	NA	NA	NA	NA	NA	NA	0.40, (1)
MTV-MOF-5-EI*	NA	NA	NA	NA	0.20, (1)	NA	NA	NA	1, (1)
MTV-MOF-5-ABC	1.0, (1)	0.052, (1)	0.52, (1)	NA	NA	NA	NA	NA	NA
MTV-MOF-5-AHI	1.0, (1)	NA	NA	NA	NA	NA	NA	0.48, (1)	0.50, (1)
MTV-MOF-5-EHI*	NA	NA	NA	NA	0.62, (1)	NA	NA	0.89, (1)	1, (1)
MTV-MOF-5-ABCD	1.0, (1)	0.12, (1)	0.56, (1)	0.40, (1)	NA	NA	NA	NA	NA
MTV-MOF-5-ACEF	1.0, (1)	NA	0.49, (1)	NA	0.22, (1)	0.62, (1)	NA	NA	NA
MTV-MOF-5-ABCHI	1.0, (1)	0.017, (1)	0.22, (1)	NA	NA	NA	NA	0.62, (1)	0.32, (1)
MTV-MOF-5-ABCGHI	1.0, (1)	0.093, (1)	0.87, (1)	NA	NA	NA	0.67, (1)	0.73, (1)	0.80, (1)
MTV-MOF-5-ABCEGHI	1.0, (1)	0.077, (1)	1.0, (1)	NA	0.69, (1)	NA	0.77, (1)	0.73, (1)	0.96, (1)
MTV-MOF-5-ABCEFGHI	1.0, (1)	0.14, (1)	0.56, (1)	NA	0.29, (1)	0.67, (1)	0.56, (1)	0.48, (1)	0.56, (1)
<i>Control of link ratio</i>									
MTV-MOF-5-ABCD-a	1.0, (1)	0.12, (1)	0.56, (1)	0.40, (1)	NA	NA	NA	NA	NA
MTV-MOF-5-ABCD-b	1.0, (0.5)	0.26, (1)	1.24, (1)	1.99, (1.5)	NA	NA	NA	NA	NA
MTV-MOF-5-ABCD-c	1.0, (1.5)	0.06, (1)	0.43, (1)	0.30, (0.5)	NA	NA	NA	NA	NA
MTV-MOF-5-ABCD-d	1.0, (1)	0.32, (1.5)	0.26, (0.5)	0.44, (1)	NA	NA	NA	NA	NA
MTV-MOF-5-ABCD-e	1.0, (1)	0.03, (0.5)	1.0, (1.5)	0.67, (1)	NA	NA	NA	NA	NA

*Numerical value of link E was normalized to 1.

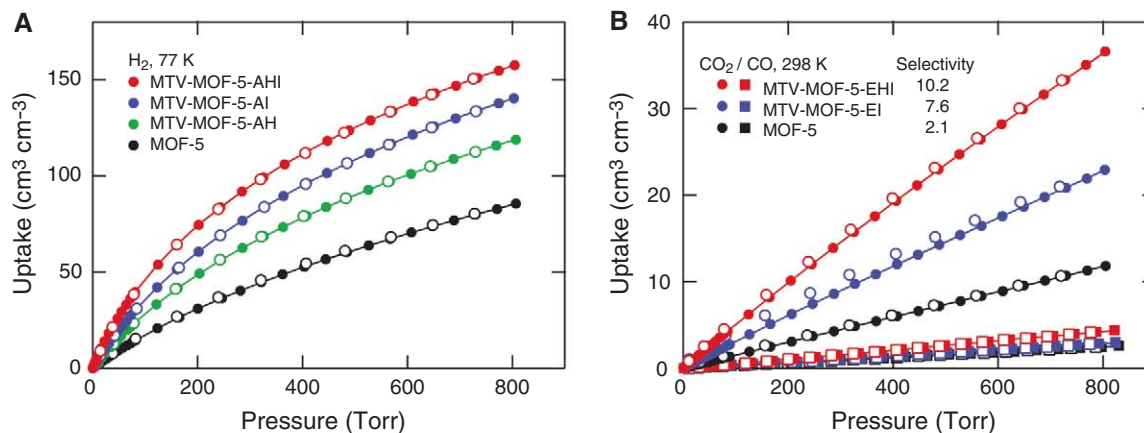


Fig. 2. (A) H₂ adsorption isotherm at 77 K of MTV-MOF-5-AH (green), -AI (blue), -AHI (red), and MOF-5 (black). (B) CO₂ (circles) and CO (squares) adsorption isotherms at 298 K of MTV-MOF-5-EI (blue), -EHI (red), and MOF-5

(black). Adsorption and desorption branches are represented by closed and open circles for CO₂ (closed and open squares for CO), respectively. Instrumental uncertainty is $\pm 5\%$.

and then the solution ¹H NMR spectra were collected on acid-digested samples of each segment of each crystal. If macroscopic domains of homogeneous links were present within a single crystal of the MTV-MOF, a different link ratio would be expected for each of the three segments of the respective parent crystal. However, the data show that the link distribution ratios are identical for each segment of the three MTV-MOFs studied (table S2), which suggests the absence of macroscopic domains. Further evidence supporting this conclusion is the absence of a narrow pore-size distribution for MTV-MOF-5-AI

as one would observe for MOF-5 (9) or any other same-link MOF, which suggests that link I is distributed throughout the pores (10). This analysis does not preclude the presence of microscopic domains where one might expect the dominance of a specific functionality (or a subset of functionalities) over the nanometer scale. We believe that the combination of the NMR experiments and pore size distribution analysis strongly support the absence of large domains (greater than a nanometer scale) of homogeneous links within the crystal. Our reasoning is that the most likely bias for one function-

ality versus another would originate from steric interactions between proximal functional groups covalently bound to the *ortho*-carbon atoms of adjacent links at the Zn₄O(CO₂)₆ vertices. Accordingly, two functionalities with disfavored interaction may not be found next to each other but would probably be accommodated separately within adjacent unit cells. These effects favor small domains (if any) and point to the possibility that the functionalities might be arranged in a specific sequence determined by the energy of functional group interactions. Evidence for this rationale is indicated by the

observed link ratios of those MTV-MOFs synthesized from an equimolar ratio of links involving the most sterically unencumbered link A, where a disproportionately higher amount of link A is retained in the resulting MTV-MOFs (Table 1).

The possible presence of distinct sequences of functionalities along the MOF backbone would inevitably lead to a complex pore environment and provide opportunities for uncovering unusual properties. Because the same-link MOF-5 structure can take up large amounts of gases (for example, H_2 , CO_2) (8, 11–13), we sought to test the MTV-MOFs in these applications and to determine whether their performance is greater than that of their constituents. In Fig. 2A, we compare the H_2 storage capacities of MTV-MOF-5-AHI, -AH, -AI, and MOF-5. The isotherms demonstrate that the uptake capacity of MTV-MOF-5-AHI is greater than that of MTV-MOF-5-AH, -AI, and -A (MOF-5) by a maximum of 84%. Similarly, an unusual increase in the selective uptake capacity of CO_2 over CO was observed: 400% better selectiv-

ity in the case of MTV-MOF-5-EHI for CO_2 compared with MOF-5 (4, 14, 15) (Fig. 2B). These findings demonstrate that the properties of MTV-MOFs are not simple linear combinations of their constituents, thus supporting the notion that the sequence of functionalities within MTV-MOF may very well be useful as code for the enhancement of a specific property or achieving a new property.

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15. Single-link MOFs of links H and I, respectively, were synthesized and found to be nonporous. Furthermore, we were unable to synthesize a single-link MOF from link E; thus, CO_2/CO separation data for these compounds was not included in our comparison.
16. This work was supported by DOE Office of Basic Energy Sciences (grant DE-FG02-08ER15935). We thank F. J. Uribe-Romo and R. Taylor for assistance and helpful discussions. MTV-MOF-5-AC and MTV-MOF-5-ACEF have been deposited into the Cambridge Crystallographic Data Centre (CCDC) under deposition numbers CCDC 747004 to 747007.

Supporting Online Material

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Figs. S1 to S52
Tables S1 to S20
References

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Break-Up of Stepped Platinum Catalyst Surfaces by High CO Coverage

Feng Tao,^{1,2} Sefa Dag,³ Lin-Wang Wang,³ Zhi Liu,⁴ Derek R. Butcher,^{1,2} Hendrik Bluhm,^{4,5} Miquel Salmeron,^{1,6*} Gabor A. Somorjai^{1,2*}

Stepped single-crystal surfaces are viewed as models of real catalysts, which consist of small metal particles exposing a large number of low-coordination sites. We found that stepped platinum (Pt) surfaces can undergo extensive and reversible restructuring when exposed to carbon monoxide (CO) at pressures above 0.1 torr. Scanning tunneling microscopy and photoelectron spectroscopy studies under gaseous environments near ambient pressure at room temperature revealed that as the CO surface coverage approaches 100%, the originally flat terraces of (557) and (332) oriented Pt crystals break up into nanometer-sized clusters and revert to the initial morphology after pumping out the CO gas. Density functional theory calculations provide a rationale for the observations whereby the creation of increased concentrations of low-coordination Pt edge sites in the formed nanoclusters relieves the strong CO-CO repulsion in the highly compressed adsorbate film. This restructuring phenomenon has important implications for heterogeneous catalytic reactions.

Industrial catalysts usually consist of small particles exposing different atomic terminations that exhibit a high concentration of step edges, kink sites, and vacancies at the edge of the facets, which are thought to be the catalytically active sites (1–3). Stepped single-crystal surfaces with well-defined surface structures can be prepared with a high density of such sites. They are models

of real catalysts because vicinal surfaces mimic closely the rough regions of the catalyst surface.

Adsorbates are known to induce structural changes, known as reconstructions in surface science. Steps are particularly notable for being modified when adsorbates bind to the surface. These are phenomena well known in vacuum surface science (4–6). The present work deals with more profound changes, driven by the formation of dense adsorbate layers. These can be formed when the adsorption energy is high—for example, when oxygen adsorbs and initiates the oxidation process. When the adsorption energy is weaker, however, dense layers can only be formed under reaction conditions of high reactant pressures near or above room temperature.

Real catalysts operate under pressures ranging from millitorr to atmospheres and from room temperature to hundreds of degrees Celsius, so

that the surfaces can easily change and adopt the structure corresponding to thermodynamic equilibrium. However, most surface science experiments are usually performed under high vacuum where the high adsorbate coverage characteristic of working catalysts cannot be attained unless the samples are kept at low temperature. These conditions will likely inhibit any restructuring process that requires overcoming of even moderate activation barriers. Thus, to understand catalytic processes at the atomic and molecular level, it is crucial to explore the structural and chemical evolution of catalyst surfaces under reaction conditions.

The limitations of traditional surface science techniques can be overcome with the use of techniques that operate under realistic conditions (7–13), including high-pressure scanning tunneling microscopy (STM) and ambient pressure x-ray photoelectron spectroscopy (AP-XPS). With these two techniques, we can image the atomic structure and identify the chemical state of catalyst atoms and adsorbed reactant molecules under realistic conditions. Here we concentrate on carbon monoxide (CO), a reactant in many important industrial catalytic processes, such as Fischer-Tropsch synthesis of hydrocarbons (14, 15), CO oxidation in automobile catalytic converters (16, 17), and degradation of Pt electrodes in hydrogen fuel cell processes (18–20).

Our studies revealed an unexpected and reversible large-scale restructuring of the surfaces of two Pt stepped surfaces, Pt(557) and Pt(332), both of which consist of six-atoms-wide terraces of (111) orientation separated by monoatomic steps of different orientation. On Pt(557) the step atoms form a (100)-type square cell, whereas on Pt(332) they form a (111)-type triangular cell (fig. S1) (21). Under high coverage of CO, these flat terraces break up into an array of nanoclusters

¹Materials Science Division, Lawrence Berkeley National Lab, Berkeley, CA 94720, USA. ²Department of Chemistry, University of California, Berkeley, CA 94720, USA. ³Computational Research Division, Lawrence Berkeley National Lab, Berkeley, CA 94720, USA. ⁴Advanced Light Source, Lawrence Berkeley National Lab, Berkeley, CA 94720, USA. ⁵Chemical Science Division, Lawrence Berkeley National Lab, Berkeley, CA 94720, USA. ⁶Department of Materials Science and Engineering, University of California, Berkeley, CA 94720, USA.

*To whom correspondence should be addressed. E-mail: somorjai@berkeley.edu (G.A.S.); mbsalmeron@lbl.gov (M.S.)

aligned along the step directions. Density functional theory (DFT) calculations were performed to help elucidate the energetics and the mechanisms underlying the restructuring from steps and flat terraces to nanoclusters.

Representative images of the Pt(557) under ultrahigh-vacuum conditions (base pressure 1×10^{-10} torr), under $\sim 5 \times 10^{-8}$ torr of CO, and under 1 torr of CO are shown in Fig. 1. When CO was introduced in the reactor cell at low pressure, the initially straight steps (Fig. 1A) became wavy (Fig. 1B). The STM images also revealed a doubling of terrace width and step height

on the Pt(557) surface (figs. S2B and S3B) (21). On Pt(332) the step edges did not increase their roughness under the same pressure conditions, a result that we ascribe to the different atomic packing of the Pt(332) and Pt(557) steps (fig. S1, A and B) (21). More dramatic effects occurred when the CO pressure was increased to 0.1 torr and higher. The terraces on Pt(557) broke down into nanoclusters about 2.2 nm by 2.1 nm in size, along [1-12] and [110] directions (Fig. 1C).

The nanoclusters have a roughly triangular shape with the vertex pointing to the lower terrace (Fig. 1, C and D). On Pt(332) the nano-

clusters are rectangular or roughly parallelogram shaped (fig. S4B) (21), a difference in geometry that is probably related to the different step structure of the surfaces. Line profiles indicate that on Pt(557) the nanoclusters in adjacent terraces are separated by 4.2 Å, i.e., two atoms in height (fig. S2D) (21), whereas neighboring nanoclusters in the same terrace are separated by ~ 2 Å deep gaps, i.e., a single atom.

XPS experiments under similar pressure conditions [Fig. 2 and fig. S5 (21)] reveal several peaks from the Pt4f core level corresponding to atoms in different coordination geometries (bulk, surface, and low-coordinated sites), and O1s peaks from CO bound to different sites (top, bridge, and low-coordinated sites). The deconvoluted Pt4f spectra (fig. S5) (21) show the increase of relative intensity of the peak component at 72.15 eV for pressures of 0.1 torr and higher (marked with red arrows), which we attribute to low-coordinated Pt atoms at cluster edges. In the O1s region in fig. S5 (21), two peaks were observed at 5×10^{-9} torr that indicate two adsorption sites on the terraces at low pressure. They are consistent with reported O1s peaks at 532.7 and 531.1 eV from CO bonded to top and bridge sites on Pt(111), respectively (22). At high pressure (≥ 0.1 torr), an increased intensity in the high-binding energy side of O1s spectra is observed.

With increasing CO coverage, the relative intensity of the O1s peak at 532.6 eV (top sites) and 531.0 eV (bridge sites) decreased, whereas there is a substantial build-up of intensity at 533.1 eV (red arrows in Fig. 2B), as the CO pressure increased to 5×10^{-1} torr. This peak is attributed to CO bound to low-coordinated Pt sites. The reversible changes of the Pt4f and O1s photoemission features as the CO pressure changed from high (5×10^{-1} torr) to low (2×10^{-8} or 3×10^{-8} torr) (Fig. 2) agrees with the reversibility of the morphological changes in the surface structure. Indeed, the nanoclusters form and disappear as the pressure is cycled between high and low values, as shown in fig. S6 (21). A similar reversible formation of nanoclusters was observed on Pt(332).

XPS allows us to determine the coverage of CO as a function of pressure (Fig. 2C). On Pt(557), the high-pressure coverage is 1.94 times that at low pressure (5×10^{-9} torr). Using the coverage of 0.5 for CO in the $c(2 \times 4)$ adsorption layer on Pt(322), Pt(355), and Pt(111) (23–25) at 5×10^{-9} torr at room temperature, we calibrated the XPS peak areas and found the coverage of CO on Pt(557) to be 0.97 at 5×10^{-1} torr. The coverage decreased to ~ 0.5 after the CO was pumped out, and increased again to 0.93 after the CO was reintroduced to 5×10^{-1} torr. Similarly, the CO coverage on Pt(332) at high pressure increased by 0.3 to 0.4 relative to that at low pressure (10^{-9} to 10^{-7} torr), and it alternately increased and decreased at high pressure and low pressure (fig. S10) (21). Such dramatic changes in real-space structure, shown by STM, and core-level binding energies, shown by XPS, between

Fig. 1. STM images of Pt(557) (A) in ultrahigh vacuum with a background pressure of 1×10^{-10} torr; (B) under $\sim 5 \times 10^{-8}$ torr of CO; and (C) under 1 torr of CO. Images are 40 nm by 50 nm in size. (D) Enlarged view of (C) showing the roughly triangular shape of the nanoclusters formed at 1 torr. Two of the clusters are marked with red lines.

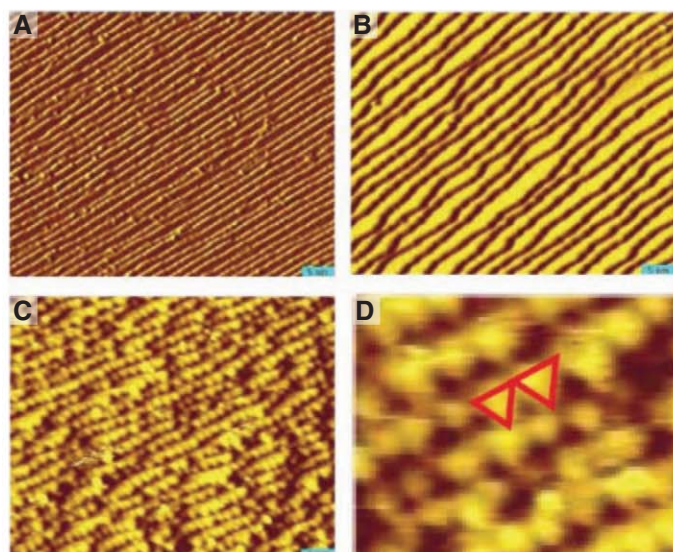
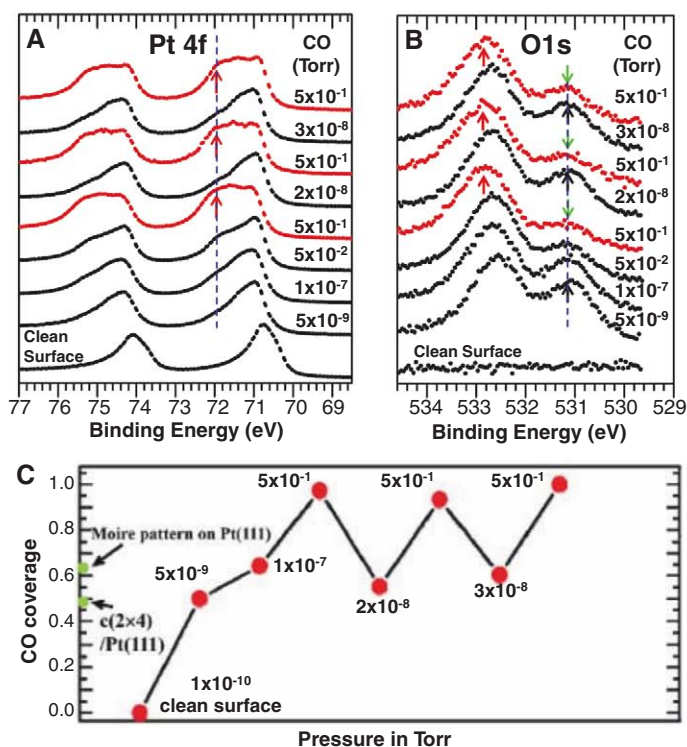


Fig. 2. Photoemission spectra of the Pt4f (A) and O1s core levels (B) acquired with 340- and 800-eV x-rays, respectively, under different CO pressures. Binding energies were referenced to the Fermi level measured under the same conditions. The red spectra were obtained at 5×10^{-1} torr. The red and green arrows in (A) and (B) mark the increase of photoemission intensity in the high-binding energy side of the Pt4f_{7/2} and the simultaneous decrease of intensity in the low-binding energy side of the O1s spectra, respectively, at high pressure. The black arrows in (B) mark the relatively larger photoemission intensity in the low-binding energy side at low pressure, compared to that at high pressure. (C) Coverage of CO on Pt(557) under different pressures as determined from the photoemission peak areas calibrated with the published 0.5 coverage of CO in the low-pressure $c(2 \times 4)$ structure at 5×10^{-9} torr.



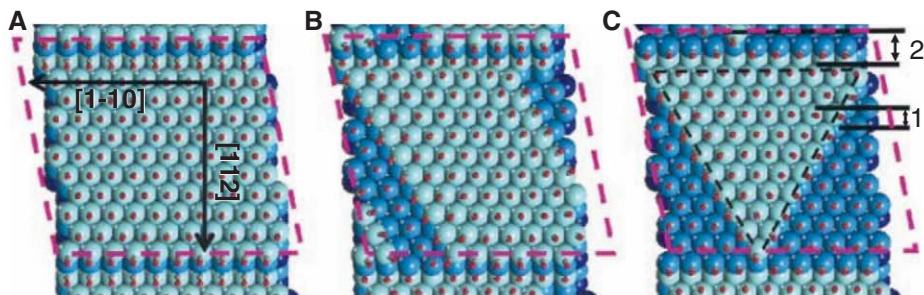


Fig. 3. Models of double-stepped Pt(557) surface covered by CO at high pressure used in the DFT calculations: (A) unrestructured terrace, (B) parallelogram-shaped nanoclusters, and (C) triangular-shaped nanoclusters. Dashed line frames in each image show the periodic supercell used in the DFT calculations. First- and second-layer Pt atoms are represented with olive green and blue balls, respectively. Red dots represent the oxygen atoms of CO molecules. In (C), 1 and 2 indicate step edge heights (one or two Pt atoms).

high and low pressure on Pt(557) and Pt(332) were not observed on Pt(111) by XPS (fig. S7) (21) or by STM (25).

The restructuring of steps upon CO adsorption at low pressure (step height doubling, kink formation) is due to changes in the energetics of the electronic and elastic step-step interaction, as has been discussed extensively (4–6). The more dramatic changes observed at higher pressure must be related to the very high coverage of CO, one molecule per surface Pt atom, which cannot be achieved under vacuum. This high density should result in a strong repulsion between CO molecules. We thus propose that the observed break-up of the surface is driven by the relaxation of the repulsive CO-CO interaction, which is facilitated by the formation of nanoclusters. Such restructuring provides a substantial increase in the number of low-coordinated edge atoms where CO molecules can tilt away from the center and thus decrease their mutual repulsion. We found also that this broken-up surface is active for CO oxidation even at room temperature (26), possibly because of a low activation barrier for CO desorption at the very high coverage.

DFT calculations were done using the experimentally observed surface structure and CO coverage as starting points. In the low-pressure regime ($<10^{-3}$ torr), a $c(2 \times 4)$ CO adlayer with a coverage of 0.5 on the flat terraces was assumed, while at 0.1 torr or higher, a full CO layer with a coverage of 1 was assumed. Calculations were carried out on a clean surface with single-atom height steps, on a surface with double terrace widths and double-atom height steps, and on a surface restructured with triangular- and parallelogram-shaped clusters. To compare energies, we used the chemical potentials of Pt and CO to account for possible differences in the number of atoms and molecules in different systems with the same surface area. Whereas the Pt chemical potential is just the bulk binding energy, the CO chemical potential was calculated from the pressure-dependent gas-phase entropy (21). On the clean Pt(557) surface, the single-atom height step structure is energetically more favorable than the double-atom height one by 0.12 eV per edge atom [table S1 (21)]. The

double-width terrace structure is energetically more favorable by 0.40 eV per edge atom upon adsorption of CO in a $c(2 \times 4)$ layer, consistent with the experimental observations (Fig. 1, A and B). In contrast to Pt(557), calculations show that on Pt(332), the surface with double-atom step heights is not more stable upon CO adsorption, again in agreement with the experimental results.

For the high-pressure (1×1) CO layer on Pt(557), we calculated the total free energy of the three structures shown in Fig. 3: a flat double terrace (A), a parallelogram created by removing two rows of Pt atoms (B), and a triangle nanocluster spanning the width of the terrace (C). For the double terrace [fig. S3B (21)], whether the CO molecules are adsorbed on Pt atoms at the bottom of the steps [A1 in fig. S3B (21)] or not gives a slightly different total energy. The calculation shows that covering the step-down side will reduce the total free energy by 0.144 eV per atom. This difference is much smaller than the adsorption energy of CO on the nanoclusters. Thus, as shown in Fig. 3, each Pt atom in the step-down side adsorbs one CO molecule in the models. The total free energies of these three systems (table S2) show that the structure with triangular nanoclusters has the lowest energy, followed by the parallelogram, and then by the flat double terrace. In the final structure after relaxation, the CO molecules adsorbed at the edge of the nanoclusters fan out considerably. This binding configuration reduces the repulsion between adjacent CO molecules and therefore decreases the energy. The increased density of low-coordinated Pt sites and coverage of CO molecules bound to such sites at high pressure (≥ 0.1 torr) (27) is consistent with the Pt4f and O1s photoemission peak structure. For example, the coverage of CO molecules bound to low-coordinated sites in the nanoclusters is $\sim 53\%$ at 0.1 torr or higher (28) and 20 to 24% at 2×10^{-8} to 1.1×10^{-7} torr (Fig. 4) (29).

The occurrence of large-scale surface restructuring of stepped Pt crystals highlights the strong connection between coverage of reactant molecules and atomic structure of the catalyst surface under reaction conditions. These results have important implications for catalysis where high pres-

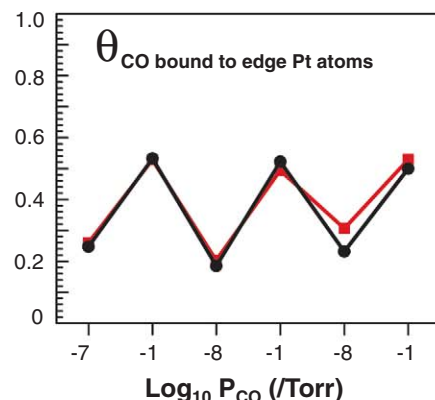


Fig. 4. Coverage of CO molecules adsorbed on low-coordination sites as a function of pressure. The red curve is the coverage calculated with the O1s peak component at 533.1 eV [D in fig. S5 (21)]. For the black curve, we used Pt4f peak component C in fig. S5 (21).

ures and high coverage of reactant molecules on the catalyst surfaces are the norm.

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- CO₂ was detected by means of a quadrupole mass spectrometer installed in the second pumping stage of the XPS lens system.
- The coverage of CO molecules bound to Pt atoms at the edge of the triangular nanoclusters used in the DFT calculation is 0.5 (Fig. 3C); the overall coverage of CO on the surface with triangular nanocluster (Fig. 3C) is 1.
- The ratio of the O1s peak area from CO bound to low-coordinated Pt atoms to the total peak area of the O1s

- peak at 0.5 torr is 0.55, and the overall CO coverage is 0.97. The coverage of CO molecules bound to low-coordinated Pt atoms is defined as the ratio of the number of CO molecules adsorbed at low-coordinated Pt atoms to the number of surface Pt atoms.
29. The ratio of the O1s peak area from CO bound to low-coordinated Pt atoms to the total peak O1s area of CO at 10^{-8} to 10^{-7} torr is 0.35 to 0.40; the overall CO coverage at this pressure is ~ 0.56 .
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Supporting Online Material

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SOM Text
Figs. S1 to S13
Tables S1 and S2
References

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Quantum-State Controlled Chemical Reactions of Ultracold Potassium-Rubidium Molecules

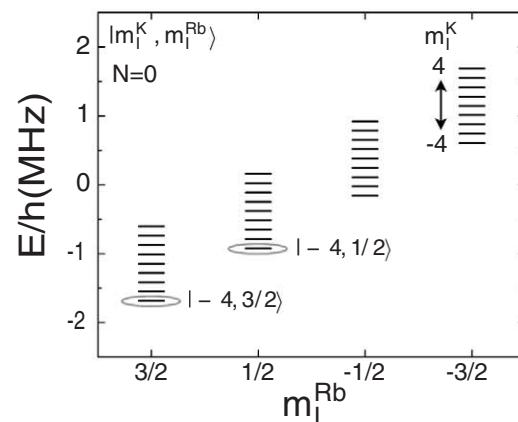
S. Ospelkaus,^{1*} K.-K. Ni,^{1*} D. Wang,¹ M. H. G. de Miranda,¹ B. Neyenhuis,¹ G. Quémener,¹ P. S. Julienne,² J. L. Bohn,¹ D. S. Jin,^{1†} J. Ye^{1†}

How does a chemical reaction proceed at ultralow temperatures? Can simple quantum mechanical rules such as quantum statistics, single partial-wave scattering, and quantum threshold laws provide a clear understanding of the molecular reactivity under a vanishing collision energy? Starting with an optically trapped near-quantum-degenerate gas of polar $^{40}\text{K}^{87}\text{Rb}$ molecules prepared in their absolute ground state, we report experimental evidence for exothermic atom-exchange chemical reactions. When these fermionic molecules were prepared in a single quantum state at a temperature of a few hundred nanokelvin, we observed p-wave-dominated quantum threshold collisions arising from tunneling through an angular momentum barrier followed by a short-range chemical reaction with a probability near unity. When these molecules were prepared in two different internal states or when molecules and atoms were brought together, the reaction rates were enhanced by a factor of 10 to 100 as a result of s-wave scattering, which does not have a centrifugal barrier. The measured rates agree with predicted universal loss rates related to the two-body van der Waals length.

Scientific interest in precisely understanding the fundamental aspects of chemical reactions and controlling their dynamic processes has stimulated pioneering work on molecular beams to study state-to-state reactions using molecular alignment, velocity selections, and angle-resolved measurement (1–5). However, substantial motional energies remained in earlier work, and thermal statistical averages were a necessary ingredient. By preparing a molecular ensemble's translational degrees of freedom in the quantum regime, we expect to develop fundamental insights into how chemical reaction processes may be precisely guided by quantum mechanics. Reaction dynamics at vanishingly low energies remain a fascinating and yet unexplored scientific realm (6). Under unprecedented energy resolution, each step of a complex reaction may be analyzed on the basis of single quantum states and single reaction channels. For example, we can study how reactivity is dictated by the quantum statistics of the molecule as a whole.

That chemical reactions could occur at ultralow temperatures seems at first glance counterintuitive. However, ultracold collisions, where particles scatter only in the partial wave with lowest angular momentum, are governed by quantum statistics and quantum threshold behaviors described by the Bethe-Wigner laws (7–9). In this regime, particles are represented by their de Broglie wavelength, which increases with reduced temperature. This wave nature of particles replaces our intuitive and classical picture of collisions. The wave manifestation of tunneling through reaction or angular momentum barriers

Fig. 1. Hyperfine structure of rovibronic ground-state $^{40}\text{K}^{87}\text{Rb}$ molecules at 545.9 G. We label the 36 nuclear spin states by their spin projections, m_I^{Rb} and m_I^{K} . The energy spacing between hyperfine states is $\sim h \times 130$ kHz for $|\Delta m_I^{\text{K}}| = 1$ and $\sim h \times 760$ kHz for $|\Delta m_I^{\text{Rb}}| = 1$. By comparison, at a temperature of 300 nK, the molecules' thermal energy is equivalent to $\sim h \times 6$ kHz, which is more than an order of magnitude smaller than the spin flip energy. In our experiments, molecules are prepared in either a single state or in a mixture of $| -4, 1/2 \rangle$ and the lowest-energy state $| -4, 3/2 \rangle$ (open ellipses).



¹JILA, NIST and University of Colorado, Department of Physics, University of Colorado, Boulder, CO 80309, USA. ²Joint Quantum Institute, NIST and University of Maryland, Gaithersburg, MD 20899, USA.

*These authors contributed equally to this work.

†To whom correspondence should be addressed. E-mail: jin@jilaui1.colorado.edu (D.S.J.); ye@jilaui1.colorado.edu (J.Y.)

may play a dominant role in dynamics, and scattering resonances can have dramatic effects on reactions (10). In addition, any barrierless chemical reactions will always take place when two reactants are sufficiently close together (11). In this case, chemical reaction rates will be determined to a large extent by collisional properties at large intermolecular separations, and thus by how the two partners approach each other. Once their separation reaches a characteristic length scale ($\sim 10a_0$, where $a_0 = 0.53 \times 10^{-10}$ m), a chemical reaction happens with a near unity probability. Therefore, chemical reactions can be surprisingly efficient even at ultracold temperatures. Indeed, this model for barrierless reactions predicts loss rates that are universal in the sense that they do not depend on the details of the short-range interactions, but instead can be estimated using only knowledge of the long-range interactions (12).

Like the case of collisions of ultracold atoms, the study of ultracold chemical reactions will play a fundamental role in advancing the field of molecular quantum gases. For example, understanding and manipulating collisions of atoms at ultralow temperatures ($< 1 \mu\text{K}$) has been crucial for the realization of quantum degenerate gases (13–15), Fermi superfluids that provide opportunities to explore the underlying connection between superconductivity and Bose-Einstein condensation (16), neutral atom-based systems for quantum information science (17–19), and strongly correlated quantum gases (20, 21). Ultracold molecules undergo a more diverse set of collisional processes, with distinct inelastic collision mechanisms arising from chemical reactions, in addition to the traditional state-changing collisions seen with ultracold atoms and highly vibrationally excited molecules (22). Furthermore,

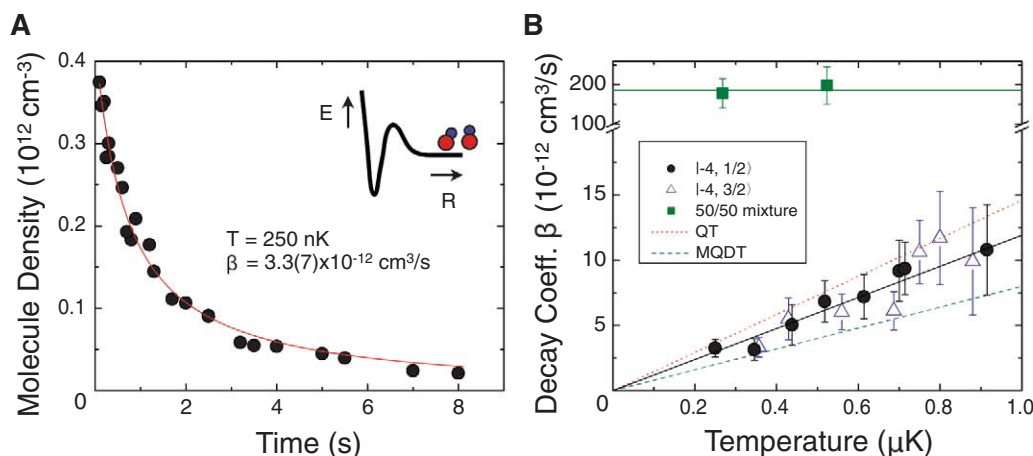
polar molecules possess anisotropic and long-range dipolar interactions that can be precisely controlled with external electric fields, with rich prospects of collisional resonances (23, 24). Experimental investigation of molecular collisions is essential for such future applications as studying anisotropic and collective behavior in quantum gases (25, 26), modeling new quantum phases and exotic many-body physics (27), implementing schemes for quantum information (28), and developing tools for precision physical and chemical measurements.

We focus here on the study of ultracold collisions, including chemical reactions, of $^{40}\text{K}^{87}\text{Rb}$ molecules, which we prepare in their lowest electronic, vibrational, rotational, and hyperfine energy state at a high phase-space density (29, 30). We find clear evidence of the essential role that quantum statistics and quantum threshold laws play in determining the rates of inelastic collisions. Our experimental observations confirm a universal loss mechanism.

Table 1. Summary of the relevant molecular energetics involved in possible chemical reactions. The binding energies are given with respect to the threshold energy for free atoms in the absence of a magnetic field. The $^{87}\text{Rb}_2$ and $^{40}\text{K}_2$ binding energies include isotope shifts from the data in the respective references. The trimer binding energies are unknown. Calculations of trimer binding energies are needed and will be important for future experiments on any bi-alkali species.

Molecule	$\nu = 0$ binding energy (D_0)	Reference
$^{87}\text{Rb}_2$	$3965.8 (\pm 0.4) \text{ cm}^{-1}$	(45)
$^{40}\text{K}^{87}\text{Rb}$	4180.417 cm^{-1}	(29)
$^{40}\text{K}_2$	$4405.389 (\pm 0.004) \text{ cm}^{-1}$	(46)
K_2Rb	Unknown	
KRb_2	Unknown	

Fig. 2. Inelastic collisions between spin-polarized (indistinguishable) or different spin-state (distinguishable) fermionic molecules in the rovibronic ground state of $^{40}\text{K}^{87}\text{Rb}$. **(A)** Sample data showing the time dependence of the molecule number density. Here the molecules are prepared in a single hyperfine state, $|{-4}, 1/2\rangle$, and the molecular density decays slowly with a rate coefficient of $3.3 (\pm 0.7) \times 10^{-12} \text{ cm}^3 \text{ s}^{-1}$ at $T = 250 \text{ nK}$. **(B)** Loss rate coefficient versus temperature. The loss rate increases linearly with temperature for spin-polarized molecules, which collide via p-wave [inset in (A)] at low temperature. Data were taken for molecules prepared in either $|{-4}, 1/2\rangle$ (solid circles) or the lowest-energy state $|{-4}, 3/2\rangle$ (open triangles). A linear fit (solid line) to the $|{-4}, 1/2\rangle$ data yields the temperature-dependent loss rate to be $1.2 (\pm 0.3) \times 10^{-5} \text{ cm}^3 \text{ s}^{-1} \text{ K}^{-1}$. For the $|{-4}, 3/2\rangle$ case, where the collisional loss can only be due to chemically reactive scattering, the loss rate is similar. The dotted and dashed lines are theoretical predictions from the QT model and MQDT, respectively. When



the molecules are prepared in a mixture of the $|{-4}, 1/2\rangle$ and $|{-4}, 3/2\rangle$ states (solid squares), we observe a temperature-independent decay rate that is 10 to 100 times that for the spin-polarized case. The error bars represent 1 SD of the decay rate coefficients arising from fluctuations of the molecular density, temperature, and fitting uncertainty of the two-body loss curves.

A prerequisite for exploring ultracold chemical reactions is a gaseous molecular sample that is sufficiently dense, ultracold, and suitable for precise control of specific quantum states (6). The starting point for this work is an ultracold trapped gas of fermionic $^{40}\text{K}^{87}\text{Rb}$ molecules prepared in a single hyperfine level of the rovibronic ground state ($N = 0, \nu = 0$ of $X^1\Sigma^+$) (29, 30). The optical trap depth is $\sim k_B \times 10 \mu\text{K}$, where k_B is Boltzmann's constant. The molecules are produced using a single step of two-photon Raman transfer from extremely weakly bound molecules at a magnetic field of $\sim 545.9 \text{ G}$. The coherent transfer is efficient and does not heat the gas, resulting in a gas of rovibronic ground-state molecules with an average number density of 10^{11} to 10^{12} cm^{-3} and a translational temperature of a few hundred nanokelvin. At this ultralow temperature, even the tiny molecular hyperfine-state energy splittings are much larger than the translational energy. Manipulation of the hyperfine states hence becomes extremely important in exploring possible collision channels. In addition, complete control over the internal quantum state of the molecules permits direct observation of the role of quantum statistics in determining the molecular interactions.

A precise measurement of the hyperfine structure and the manipulation of individual hyperfine state populations in the $X^1\Sigma^+$ ground state of $^{40}\text{K}^{87}\text{Rb}$ were reported in (30). The $X^1\Sigma^+$ state has zero total electronic angular momentum, so that the hyperfine structure is basically the Zeeman effect of the nuclear spins $I^K = 4$ and $I^{\text{Rb}} = 3/2$ (12, 31) at the applied magnetic field. The hyperfine structure is depicted in Fig. 1, where a total of 36 states are labeled by their projections of the individual nuclear spins, m_I^{Rb} and m_I^K . For the current study, we produce molecules either in a single spin state ($|m_I^K, m_I^{\text{Rb}}\rangle$) or in a mixture of two spin states. The hyperfine states used are an excited state $|{-4}, 1/2\rangle$ and the lowest-energy spin state $|{-4}, 3/2\rangle$; these are marked by the two

ellipses in Fig. 1. The $|{-4}, 1/2\rangle$ state is populated directly by the two-photon Raman transfer starting from the weakly bound molecules (29, 30). The spin state of these molecules can be further manipulated coherently. For example, the entire $|{-4}, 1/2\rangle$ population can be transferred into the lowest hyperfine state, $|{-4}, 3/2\rangle$, using two successive π pulses through a rotationally excited $N = 1$ intermediate level. The $N = 1$ level has strong nuclear electric quadrupole interactions that couple rotations with nuclear spins, enabling nuclear spin flips (30, 31). We can probe molecules in any particular hyperfine state by reversing the entire transfer process and putting the population back into the initial weakly bound state. We then use high-signal-to-noise ratio absorption imaging to measure the molecular gas number and temperature.

The ability to control molecular internal states including the electronic, vibrational, rotational, and nuclear spin degrees of freedom, and in particular the possibility of preparing them in the lowest-energy state, allows us to probe inelastic collisions in a way that limits unwanted loss mechanisms and reduces ambiguities in the identification of possible chemical reaction channels. This is crucial because it is difficult in the ultracold gas experiment to find probes for the direct observation of reaction products. In Table 1, we consider the binding energies for various types of molecules made from different combinations of ^{40}K and ^{87}Rb atoms. These energy estimates allow us to assess whether a specific two-body reaction process is endothermic or exothermic. We note that all results reported here were obtained in the absence of any external electric field, and hence the effective molecular dipole moment in the lab frame is zero.

To probe the quantum nature of ultracold molecular collisions and chemical reactions, we begin by preparing the KRb molecules in a single nuclear spin state of the rovibronic ground state.

All unpaired atoms that remain after the initial stage of the molecular creation process are selectively removed from the optical trap via resonant light scattering (32). From an argument based on the energetics summarized in Table 1, the molecule-molecule collisions have a possible exothermic chemical reaction, namely $\text{KRb} + \text{KRb} \rightarrow \text{K}_2 + \text{Rb}_2$, which releases $\sim 10 \text{ cm}^{-1}$ of kinetic energy. The reactions $\text{KRb} + \text{KRb} \rightarrow \text{K}_2\text{Rb} + \text{Rb}$ and $\text{KRb} + \text{KRb} \rightarrow \text{KRb}_2 + \text{K}$ could also be exothermic. All of these reactions require breaking and making molecular bonds. If the KRb molecules are prepared in an excited hyperfine state, spin relaxation to a lower hyperfine state provides an additional inelastic scattering mechanism.

The quantum statistics of the molecules plays an essential role in collisions at a temperature of a few hundred nanokelvin, where collisions with large-impact parameters and correspondingly large centrifugal barriers are frozen out and the collisions are typically dominated by a single partial wave with orbital angular momentum quantum number $L = 0$ (s-wave) or $L = 1$ (p-wave). Our KRb molecules are fermions, and therefore the total wave function describing a $\text{KRb} + \text{KRb}$ collision is antisymmetric with respect to molecular exchange. For spin-polarized molecules all prepared in exactly the same internal quantum state, the p-wave is the lowest-energy symmetry-allowed collision channel. The height of the centrifugal barrier for the $L = 1$ KRb-KRb collisions is $k_B \times 24 \mu\text{K}$ (33, 34). This barrier height is more than an order of magnitude larger than $k_B T$, where T is the translational temperature of the molecular gas. Thus, collisions of spin-polarized molecules are expected to proceed predominantly via tunneling through the p-wave barrier. Note that T is greater than 1.4 times the Fermi temperature. If two molecules make it through the barrier to short range, chemical reactions or hyperfine state-changing collisions can take place, leading to a loss of the entrance channel population. We note that even a single nuclear spin flip corresponds to a released quantity of energy that is above the trap depth, and this would contribute to loss of trapped molecules.

The quantum nature of the collisions can be seen in the temperature dependence of loss rates. The Bethe-Wigner threshold law predicts that the p-wave inelastic/reactive collision rate should be linear in temperature ($\propto T$). To look for this behavior, we first prepared spin-polarized molecules in the single hyperfine state $|-4, 1/2\rangle$ for various values of T ranging from 200 to 900 nK (35). The temperature is measured from the expansion energy of the molecular gas after releasing it from the optical trap. For each initial temperature, we observed the time-dependent molecular loss (36) and extracted a two-body loss rate β (which is twice the collisional event rate) by fitting the measured decay of the molecular gas density n versus time t (Fig. 2A) to

$$\frac{dn}{dt} = -\beta n^2 - \alpha n \quad (1)$$

Here, the first term on the right accounts for number loss and the measured β can be compared to theoretical predictions. The second term accounts for density change due to heating of the trapped gas during the measurement. Within a single measurement, we observe an increase in temperature that is at most 30%. In the analysis for each data set, we fit the measured temperature to a linear heating rate and obtain a constant slope c . In Eq. 1, we then use $\alpha = (3/2)[c/(T + ct)]$, where T is the initial temperature. At our lowest temperature of 250 nK, the heating was $7 (\pm 1) \text{ nK s}^{-1}$ and $\beta = 3.3 (\pm 0.7) \times 10^{-12} \text{ cm}^3 \text{ s}^{-1}$ (Fig. 2A). The measured dependence of β versus T is summarized in Fig. 2B (solid circles). Here, we fit the data to a power law $\beta(T) \propto T^L$ and find that $L = 1.1 (\pm 0.2)$, which agrees with the predicted p-wave threshold law. This result demonstrates that indistinguishable $^{40}\text{K}^{87}\text{Rb}$ molecules at ultralow temperatures collide via tunneling through a p-wave barrier followed by an inelastic collision in the short range. A linear fit to the data ($L = 1$) yields a slope of the decay rate coefficient of $1.2 (\pm 0.3) \times 10^{-5} \text{ cm}^3 \text{ s}^{-1} \text{ K}^{-1}$.

We repeated this measurement for molecules in the lowest hyperfine state $|-4, 3/2\rangle$ (open triangles in Fig. 2B). The data again show $\beta \propto T$

with a slope of $1.1 (\pm 3) \times 10^{-5} \text{ cm}^3 \text{ s}^{-1} \text{ K}^{-1}$, similar to that measured for molecules in the $|-4, 1/2\rangle$ state. However, in the case of $|-4, 3/2\rangle$ molecules, hyperfine state-changing collisions are no longer possible and the only possible loss channels are the chemical reactions discussed above. Thus, we find that the rate of chemical reactions is determined by the p-wave angular momentum barrier, and the chemical reaction barrier must be below the collision energy. This suggests that these reactions are barrierless and can thus occur freely at ultralow temperatures. Meanwhile, the fact that the same loss rate is observed for both $|-4, 1/2\rangle$ and $|-4, 3/2\rangle$ state molecules suggests that chemical reactions dominate the loss in these ground-state molecular collisions.

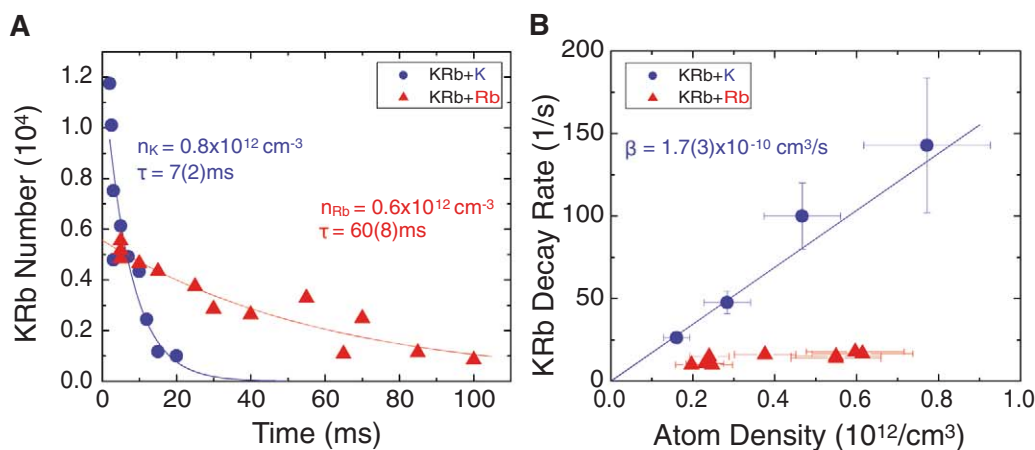
To understand the loss rates, we use two models: a simple quantum threshold model (QT), and a model that uses the formalism of multi-channel quantum defect theory (MQDT). In the QT model, the loss rate for collisions with energy equal to or above the height of the p-wave barrier is determined by the Langevin capture rate (37), which assumes that the probability for chemical reactions and/or hyperfine state-changing collisions is unity. For all collision energies below the height of the p-wave barrier, we assume in this model that the loss follows the Bethe-Wigner threshold laws (7, 8). Using this assumption, we obtain a simple analytical expression for the p-wave loss rate coefficient of two indistinguishable molecules, which scales linearly with T (38):

$$\beta = \frac{\pi}{4} \left(\frac{3^{17} \mu^3 C_6^3}{\hbar^{10}} \right)^{1/4} k_B T \quad (2)$$

where μ is the reduced mass and $\hbar$ is Planck's constant divided by 2π . Using a van der Waals dispersion coefficient of $C_6 = 16130 \text{ a.u.}$ for KRb-KRb with an uncertainty of $\pm 10\%$ (33, 34), the slope of the rate coefficient is predicted to be $1.5 (\pm 0.1) \times 10^{-5} \text{ cm}^3 \text{ s}^{-1} \text{ K}^{-1}$, which agrees well with the experimental measurement.

In the second model, the loss rate coefficient is found directly by calculating the

Fig. 3. Collisions of atoms and molecules in their lowest-energy internal states. **(A)** Sample decay curves for the molecular number when subject to collisions with K atoms (circles) and with Rb atoms (triangles). The atom numbers are 5 to 15 times the molecule numbers. The reduced initial molecule number for $\text{KRb} + \text{Rb}$ is due to the collisional quenching of the initial weakly bound KRb molecules with Rb (42) before the two-photon Raman process that transfers the molecules down to the rovibronic ground state. **(B)** Dependence of molecule loss rate on atomic gas density. We observed strong molecule loss with $\beta = 1.7 (\pm 0.3) \times 10^{-10} \text{ cm}^3 \text{ s}^{-1}$ for $\text{KRb} + \text{K}$ collisions and suppressed loss of KRb for $\text{KRb} + \text{Rb}$ collisions. The error bars are 1 SD of decay rates and atomic densities.



quantum tunneling rate through the p-wave barrier (39). This calculation gives $\beta = 0.8 (\pm 0.1) \times 10^{-5} \text{ cm}^3 \text{ s}^{-1} \text{ K}^{-1}$, which agrees with the experiment within mutual uncertainties. This β can also be derived analytically from the properties of the long-range potential to give $\beta = (11.48 \bar{a})^3 (k_B T/h)$, where $\bar{a} = 0.4778 (2\mu C_6/\hbar^2)^{1/4} = 6.3 \text{ nm}$ is the characteristic length of the van der Waals potential (12). This fully quantum calculation can be put in the same form as the QT model (Eq. 2) and gives a β that is smaller by a factor of 0.528. With these simple theories, the agreement with our molecule-molecule collisional loss measurements suggests that the chemical reaction rates are strongly influenced by the long-range interactions. This observation opens intriguing control possibilities because the long-range interaction can be controlled by selecting quantum states and tuning collision energies via applied electric and magnetic fields.

Reaction rates should be markedly different if molecules are prepared in a mixture of different hyperfine states as s-wave scattering becomes allowed. We measured the inelastic collision rates for rovibronic ground-state molecules that were prepared in a roughly 50-50 incoherent mixture of the two hyperfine spin states $|-4, 3/2\rangle$ and $|-4, 1/2\rangle$. The time-dependent number density of trapped molecules was measured for both spin states. We observed the same loss rate for both states, consistent with loss due to collisions between distinguishable molecules in different spin states. The rate coefficient is determined to be $1.9 (\pm 0.4) \times 10^{-10} \text{ cm}^3 \text{ s}^{-1}$, independent of temperature (solid squares in Fig. 2B). In comparison to our measurements for p-wave collisions between spin-polarized molecules, the s-wave collision rate between molecules in different hyperfine states is higher by a factor of 10 to 100 for a similar temperature range.

The MQDT model can also be used to estimate collision rates where the dominant collision channel is s-wave. Here, the relevant length scale is determined by the inter-molecular van der Waals potential without any angular momentum barrier (40). We assume that when the molecules approach each other within the van der Waals length $\bar{a}$, chemical reactions take place and remove these entrant molecules with a near-unity probability. The universal loss rate coefficient, $\beta = 2(h/\mu) \bar{a}$ (12), predicts a β value of $0.8 \times 10^{-10} \text{ cm}^3 \text{ s}^{-1}$, which is a factor of 2 lower than the experimentally observed value. This difference suggests that short-range physics may play some role in the loss dynamics. An enhancement in the rate coefficient (up to the energy-dependent unitarity limit, which is $4 \times 10^{-10} \text{ cm}^3 \text{ s}^{-1}$ for a gas at 400 nK) is possible if there is a partial reflection of the colliding species back into the entrance channel (39). The reflected amplitude interferes with the incoming amplitude and can either increase or decrease the rate coefficient from its "universal" value. Additional theory and experiment are needed to explore this possibility.

In addition to molecule-molecule reactions, the relatively long lifetime of a pure gas of spin-polarized molecules in the optical trap ($\sim 1 \text{ s}$) affords time to look for chemical reactions between atoms and molecules. The MQDT model described above can also predict the atom-molecule reaction rates determined by the long-range physics for the universal loss mechanism. To prepare the atom-molecule mixture, we control the atom density by selectively removing or heating unpaired atoms after the initial molecule creation (41). For these experiments, we typically work with an atom number about 5 to 15 times the molecule number. All atoms and molecules are prepared in their lowest-energy states at 545.9 G. Specifically, K atoms are in their $|F=9/2, m_F=-9/2\rangle$ state, Rb in $|F=1, m_F=1\rangle$, and KRb in $|-4, 3/2\rangle$. Here F is the total atomic spin and m_F is the spin projection. We performed two separate experiments, one with K and KRb and the second with Rb and KRb, at a temperature less than $1 \mu\text{K}$. In both cases, the background of atoms in the other, undesired, species was less than 1000, corresponding to a density below $5 \times 10^9 \text{ cm}^{-3}$. Because both the atoms and the molecules are prepared in their lowest-energy states, trap loss due to inelastic spin-changing collisions is not possible. However, from Table 1, we do expect loss from chemical reactions for the exothermic reaction $\text{K} + \text{KRb} \rightarrow \text{K}_2 + \text{Rb}$, whereas the endothermic $\text{Rb} + \text{KRb} \rightarrow \text{Rb}_2 + \text{K}$ should be forbidden.

For each experiment, we measured the time dependence of the trapped molecule population. Typical molecular loss curves are shown in Fig. 3A. To extract the inelastic collision rate, we assume that the atom number density is constant. (This is approximately true because the number of atoms is much larger than the number of molecules.) The trapped molecule number should then decay as

$$\frac{d}{dt} N_{\text{molecule}} = -\beta \times N_{\text{molecule}} \times n_{\text{atom}} \quad (3)$$

where N_{molecule} is the number of molecules, n_{atom} is the atomic density, and β is the inelastic rate coefficient. We can then extract β via an exponential fit, $\exp(-\beta \times n_{\text{atom}} \times t)$. As can be seen in Fig. 3B, in general we find that the molecules are lost from the trap at a much faster rate when K atoms are present than when a similar density of Rb is present.

To see if the molecule loss arises from atom-molecule collisions, we measure the dependence of the loss rate on the atom gas density. For the case of KRb + K, we observe a clear linear dependence on the atomic density and extract an inelastic collision rate coefficient of $1.7 (\pm 0.3) \times 10^{-10} \text{ cm}^3 \text{ s}^{-1}$. This rate coefficient (due to chemical reactions) can again be predicted from the van der Waals length characterizing the long-range part of the potential between the collision partners. Using methods similar to those used for KRb + KRb, Kotochigova has calculated C_6 for KRb + K to be $7020 (\pm 700) \text{ a.u.}$ (34), which gives

a predicted reaction rate of $1.1 \times 10^{-10} \text{ cm}^3 \text{ s}^{-1}$. Again we find that the MQDT value is close but somewhat lower than the measured value; this result further solidifies the importance of long-range quantum scattering in the inelastic process.

In contrast, for KRb + Rb collisions, the density dependence of the loss rate is not obvious (Fig. 3B). Nevertheless, we again fit the dependence as linear and obtain an upper limit for the rate coefficient of $0.13 (\pm 0.04) \times 10^{-10} \text{ cm}^3 \text{ s}^{-1}$, which is one order of magnitude smaller than what we measure for KRb + K. Our measurement is consistent with the fact that for KRb + Rb, there is no two-body chemical reaction pathway. A possible mechanism for the residual nonzero rate coefficient for KRb + Rb would be collisions of ground-state molecules with undetected molecules in high-lying vibrationally excited states. These contaminant molecules could be produced by inelastic collisions of Rb atoms with our weakly bound KRb molecules (42) before the two-photon Raman process that produces ground-state molecules. Another possible loss mechanism is Rb + Rb + KRb three-body inelastic collisions. Further experiments will be needed to check these possibilities. We note that this suppressed loss rate is observed only when both KRb and Rb are prepared in their lowest-energy internal states at 545.9 G. If either the molecules or the atoms are in an excited hyperfine state, then the observed inelastic rate coefficient rises again to the order of $10^{-10} \text{ cm}^3 \text{ s}^{-1}$. Therefore, for practical reasons, removing Rb atoms is important for creating a long-lived sample of ground-state KRb molecules.

Together the studies presented here show that we have observed barrierless chemical reactions in the short range, with the rates determined by long-range scattering dynamics dictated by quantum statistics, angular momentum barriers, and threshold laws. We see that a change as seemingly insignificant as flipping a single nuclear spin dramatically changes the rate of molecular collisions. A pure gas of spin-polarized KRb molecules is long-lived in an optical trap (surviving for a time on the order of 1 s) whereas a spin mixed KRb sample decays 10 to 100 times as quickly. Our results clearly show that chemical reactions can proceed with high rates in the ultracold regime.

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32. Residual unpaired Rb atoms are removed immediately after creating weakly bound molecules. We use a sequence that includes adiabatic rapid passage using frequency-swept microwaves for transitioning Rb atoms from $|F = 1, m_F = 1\rangle$ to $|2, 2\rangle$ and then a pulse of laser light resonant with the cycling transition from $|2, 2\rangle$ to $|F' = 3, m_{F'} = 3\rangle$ to heat the atoms out of the trap. The sequence is performed three times within a time span of 1 ms. Unpaired K atoms are removed immediately after the molecules are transferred into the rovibronic ground state. We apply a 0.5-ms pulse of light on the cycling transition $|F = 9/2, m_F = -9/2\rangle$ to $|F' = 11/2, m_{F'} = -11/2\rangle$. Any trapped atoms left after this removal are below our detection limit, which is about 1000 atoms.
33. The height of the p-wave barrier is determined by the molecule-molecule long-range potential, namely $[\hbar^2 L(L+1)/2\mu R^2] - (C_6/R^6)$, for $L = 1$ and $C_6 = 16,130 E_h \times a_0^6$ (a.u.), where $E_h = 4.36 \times 10^{-18}$ J and $a_0 = 0.53 \times 10^{-10}$ m.
34. The calculated van der Waals dispersion coefficients, C_6 , used here for KRb + KRb, KRb + K, and KRb + Rb potentials were provided to us by S. Kotochigova. This was done using nonrelativistic ab initio calculations of the dynamic polarizability of KRb in the standard formula for the van der Waals coefficient (43, 44), including the contributions from the spectrum of electronically excited states and the ground-state rovibrational levels.
35. We control the molecular gas temperature by varying the initial temperature of our Rb and K atom gases during forced evaporative cooling.
36. Loss due to blackbody radiation driving either vibrational or rotational excitations is negligible, as this is expected to occur on the time scale of 10^5 s.
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41. To remove selective amounts of K, we partially transfer the population of K atoms from $|F = 9/2, m_F = -9/2\rangle$ to $|9/2, -5/2\rangle$ using two successive radio-frequency (rf) pulses. We then clean out K atoms in the $|9/2, -9/2\rangle$ state using resonant light as in (32). Once these atoms are heated out of the trap, we again use rf pulses to spin-flip all K $|9/2, -5/2\rangle$ atoms back down to $|9/2, -9/2\rangle$. To selectively vary the density of Rb, we perform a complete spin flip from $|1, 1\rangle$ to $|2, 2\rangle$ and then turn on resonant light for a duration between 1 and 10 μ s. The resonant light both removes Rb atoms and heats the remaining gas. After the resonant light pulse, Rb atoms are transferred back to the lowest hyperfine state $|1, 1\rangle$. We then apply an additional resonant light pulse to remove any residual Rb in $|2, 2\rangle$, thus ensuring that all Rb atoms are in the $|1, 1\rangle$ state.
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Low-Frequency Modes of Aqueous Alkali Halide Solutions: Glimpsing the Hydrogen Bonding Vibration

Ismael A. Heisler and Stephen R. Meech*

The solvation of ions in aqueous media is a fundamental process in biology and chemistry. Here, we report direct time-domain observations of the hydrogen bond vibrational mode formed between a halide ion (chloride, bromide, or iodide) and the surrounding water molecules. The frequency of the hydrogen bond mode is sensitive to both the atomic weight and the concentration of the ion. The peak frequencies fall in the 125 to 175 wave-number range, a spectral region accessed through time-domain polarization-resolved coherent Raman scattering using a diffractive optic method. The polarized Raman response observed is discussed in terms of the structure of the anion's solvation shell and modeled through calculations on water chloride clusters.

Aqueous solvation of ions is a central process in many chemical and biological reactions. It plays a critical role in determining acid-base equilibria (1), interface structure (2), and ion transport in electrolyte solutions (3) and across membranes (4). Aqueous solutions of alkali halide salts present the archetypal example of solvation and have consequently been studied in great detail through both experiment and simulation (5). Important progress in understanding the microscopic structure and dynamics

of solvated ions has recently been made through ultrafast vibrational spectroscopy (6–9) and molecular dynamics simulation (10–14). Most of these experiments focused on the readily observable infrared (IR)-allowed OD stretch of monodeuterated water (HOD) in aqueous solutions of alkali halides. The vibrational relaxation and molecular orientational relaxation times observed are significantly slower compared with the same measurements for HOD in pure H₂O and become slower still with increasing concentration of the electrolyte (7, 9). Two-dimensional IR spectroscopy on the OD stretch reveals further information on the molecular dynamics, suggesting a hierarchy of relaxation times. The fastest component arises from local

fluctuations in the length of the OD $\cdots$ X[−] hydrogen bond (H-bond), where X[−] is the anion. The intermediate (subpicosecond) and longer (picosecond) relaxation components, which increase with increasing halide ion concentration, reflect the transition from local H-bond fluctuations to global reorganization and the reorganization of H-bond networks, respectively (9). From these studies of the OD mode, inferences on the structure and dynamics of the OD $\cdots$ X[−] H-bonds are drawn. However, the H-bond mode itself is not well characterized, and a more complete description of it will help to refine the analysis and simulation of aqueous solvation dynamics.

Here, we report direct time-domain experimental observations of the spectra of the OH $\cdots$ X[−] hydrogen bond through measurements of the low-frequency isotropic Raman response. To access the low-frequency region of the spectrum, where H-bond modes are expected to appear, we probed in the time domain the decay of polarization induced in the sample by an ultrafast pump pulse (15). In a two-beam pump-probe configuration, this experiment measures the transient optical Kerr effect, which, when combined with heterodyne detection and Fourier transform analysis, yields the Raman spectral density with excellent signal-to-noise ratio in the 0- to 500-cm^{−1} region (16). In the present experiments, we exploited a four-beam transient grating diffractive optic geometry, described in detail by Goodno *et al.* (17). Essentially, pump and probe beams are focused onto a diffractive optic element to

School of Chemistry, University of East Anglia, Norwich NR4 7TJ, UK.

*To whom correspondence should be addressed. E-mail: s.meech@uea.ac.uk

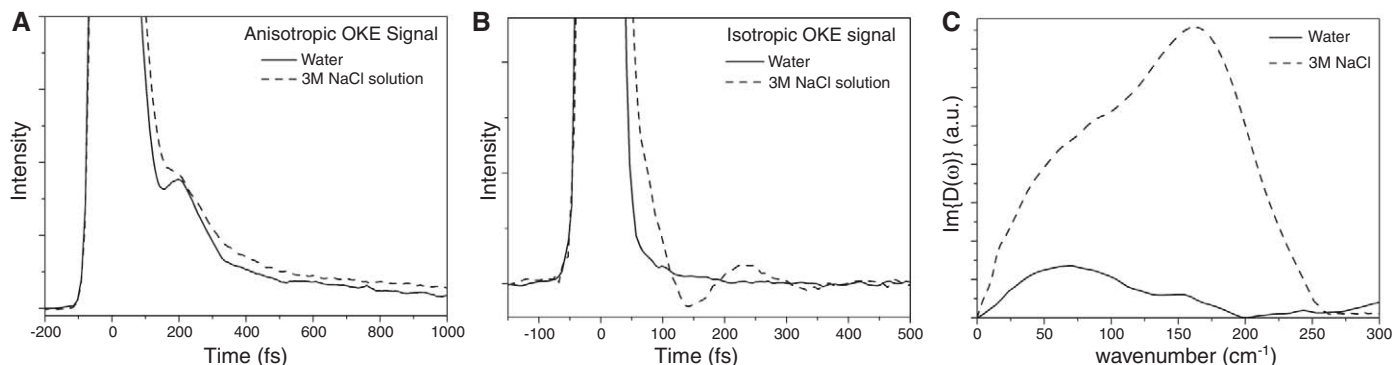


Fig. 1. Measurements of the anisotropic (A) and isotropic (B) third-order responses for water (solid lines) and 3M NaCl solutions (dashed lines). After performing a Fourier transform of the isotropic signal (B) and taking the imaginary part, the corresponding spectral densities (C) are obtained.

create two identical pairs of pulses that are brought to a common focus in the sample. The pump-pulse pair generates a transient polarization grating in the sample from which one of a pair of time-delayed probe pulses scatters a signal in a direction that is temporally and spatially overlapped with the second of the probe pulses. Constructive or destructive interference between this third-order nonlinear optical signal and the transmitted probe pulse yields in-phase and out-of-phase components, respectively, with high sensitivity. This geometry has several advantages over the two-beam geometry, and here we exploited in particular its ability to measure the polarizability relaxation with arbitrary pump-probe polarizations (17). By selecting appropriate relative pump and probe polarizations (15), the anisotropic response, $R_{\text{aniso}}(t)$, and the isotropic response, $R_{\text{iso}}(t)$ are measured. Only these two measurements are required to completely characterize the third-order response of an isotropic medium (18). In a two-beam experiment, only the $R_{\text{aniso}}(t)$ response can be determined with high accuracy.

These two measurements are shown in Fig. 1, A and B, for water and 3 M NaCl solution, normalized to the intense response at $t = 0$ (which arises from the electronic hyperpolarizability and thus contains no information on molecular dynamics). The isotropic and anisotropic measurements [which are formally equivalent to the polarized and depolarized Raman spectral density in the frequency domain (19)] yield fundamentally different information on spectroscopy and dynamics of the liquid phase (20, 21). In terms of molecular vibrational modes, the isotropic spectrum reveals symmetric stretching vibrations, rather than the depolarized modes, such as deformation vibrations and asymmetric stretches, which appear in the anisotropic response. In addition to molecular vibrations, the low-frequency Raman spectrum also contains information on molecular reorientation and intermolecular interactions (16). Molecular reorientation appears in $R_{\text{aniso}}(t)$ only and is suppressed in $R_{\text{iso}}(t)$, which contains only contributions from intermolecular (interaction induced) components of the sample polarizability (20).

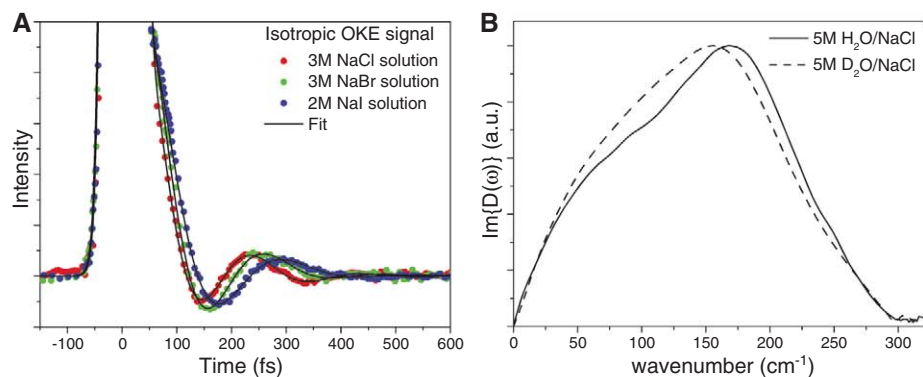


Fig. 2. (A) The isotropic signal for 3M NaCl solution (red dot), 3M NaBr solution (green dot), and 2M NaI solution (blue dot). Data were fit (black line) to a single exponential (time constant 56 ± 8 fs for all data) plus a damped harmonic oscillation with damping constant of 90 ± 10 fs for all data and frequencies: $\nu_{\text{NaCl}} = 168 \pm 4 \text{ cm}^{-1}$; $\nu_{\text{NaBr}} = 150 \pm 5 \text{ cm}^{-1}$; $\nu_{\text{NaI}} = 132 \pm 6 \text{ cm}^{-1}$. (B) Isotope effect on the isotropic spectral density for 5 M solutions of NaCl in water (solid line) and deuterated water (dashed line). The isotope shift is $12 \text{ cm}^{-1} \pm 3 \text{ cm}^{-1}$. The spectra were obtained from a direct Fourier transform of the experimental data (the time-domain fits to a single damped oscillator are shown in fig. S5).

The $R_{\text{iso}}(t)$ data are shown in Fig. 1B for pure water and a 3 M NaCl solution. For pure water, the isotropic response is an exponential decay with a 56 ± 8 fs time constant, whereas for the NaCl solution a prominent strongly damped oscillatory response is superimposed on the 56 fs exponential decay. Fourier transformation of these data to the frequency domain (Fig. 1C) reveals that aqueous NaCl has a broad but well-defined mode with a peak frequency of $168 \pm 4 \text{ cm}^{-1}$. There is no corresponding mode in the pure water spectrum. Because no vibrations can be associated with the monoatomic ions, this band represents a vibration associated with a water-ion interaction. The same measurement was made for aqueous 3 M KCl, and an identical spectrum was recovered (fig. S4), which establishes this mode as arising from an interaction between the anion and water.

The isotropic response of pure water at low frequency (Fig. 1C) has been reported previously (20), with lower signal-to-noise ratio. The signal enhancement associated with the diffractive optic experiment allows us to confirm the appearance of a broad mode in the polarized Raman spectrum around 60 cm^{-1} and therefore to establish that the depolarization ratio ρ is below 0.75,

a point that has previously been unclear (20). A transition at 60 cm^{-1} has been reported in the depolarized Raman spectrum and was assigned to a bending mode of water molecules linked by hydrogen bonds. A depolarized 180-cm^{-1} mode was also observed and assigned to an H-bond translational (stretching) mode (22). Both of these modes are delocalized over several water molecules (23). The latter mode is responsible for the oscillation seen in the $R_{\text{aniso}}(t)$ for pure water (Fig. 1A) but is absent from the isotropic response (Fig. 1, B and C). This 180-cm^{-1} depolarized mode is attenuated and shifted to lower frequency in NaCl solutions (Fig. 1A and fig. S7), as described elsewhere (22, 24). The appearance of this mode only in $R_{\text{aniso}}(t)$ suggests an assignment to intermolecular translational modes between water molecules, involving interactions of the isotropic components of the molecular polarizability, because this intermolecular interaction will contribute to $R_{\text{aniso}}(t)$ but not to $R_{\text{iso}}(t)$ (20, 21); for water, the isotropic part of the polarizability has previously been predicted to be the dominant one, consistent with this assignment (21).

We next explored the isotropic response of heavier halide salt solutions. The $R_{\text{aniso}}(t)$ responses were also measured for most solutions

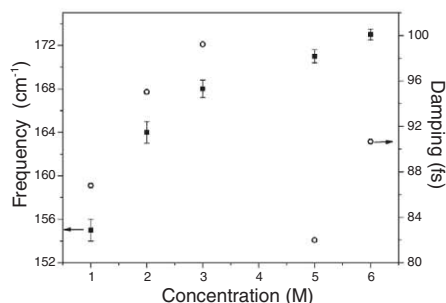


Fig. 3. Parameters resulting from fitting the concentration dependence of the isotropic response of 1 to 6 M NaCl solutions with a single damped oscillator. Black squares are the recovered frequencies and open dots the damping constants. Error bars reflect the standard deviation of 3 separate experiments.

(figs. S6 and S7), and they agree well with the earlier frequency domain data reported by Tominaga and co-workers (24). The $R_{\text{iso}}(t)$ data for 3 M sodium chloride, bromide, and iodide solutions were fit in the time domain to an exponential decay plus a single damped oscillator, $R_{\text{iso}}(t) = \exp(-t/\tau_r) + \exp(-t/\tau_0)\sin \omega t$, in which τ_r and τ_0 are the exponential and oscillator damping constants, respectively, and ω is the oscillator frequency (Fig. 2). The quality of the fits is good and shows that with increasing size and mass of the ion the damped oscillation shifts to lower frequency (legend to Fig. 2A) while its damping rate remains essentially constant at $\tau_0 = 90 \pm 10$ fs. For all solutions, a τ_r value of 56 ± 8 fs is recovered, the time constant associated with pure water. The increasing amplitude of this low-frequency bending mode with increasing anion concentration suggests that the transition undergoes an intensity enhancement in alkali halide solutions. The effect of anion mass supports an assignment to an $\text{OH}\cdots\text{X}^-$ H-bond. Confirmation of this assignment comes from measurements in deuterated water, which shift the mode to lower frequency by 12 cm^{-1} ($\pm 3 \text{ cm}^{-1}$) (Fig. 2B and fig. S5). In addition, 168 cm^{-1} is close to the 210-cm^{-1} frequency reported for a chloride-water complex in the gas phase (25).

The experimentally determined parameters for the oscillation in $R_{\text{iso}}(t)$ may be discussed in the light of molecular dynamics simulations of the spectral diffusion and vibrational relaxation of the OH stretch around iodide (12) and chloride ions (11). Both simulations report an oscillatory subpicosecond component assigned to dynamics associated with the $\text{OH}\cdots\text{X}^-$ H-bond, leading to frequencies of 100 cm^{-1} and 145 cm^{-1} for I^- and Cl^- , respectively. These frequencies are in reasonable agreement with the present observations (Fig. 2). The 145-cm^{-1} frequency was assigned to an H-bond stretching vibration (11). The 90-fs damping constant, τ_0 , recovered from the fit correlates well with the 100- to 200-fs evolution observed in both simulations and measurements of spectral diffusion (9, 11, 12). This

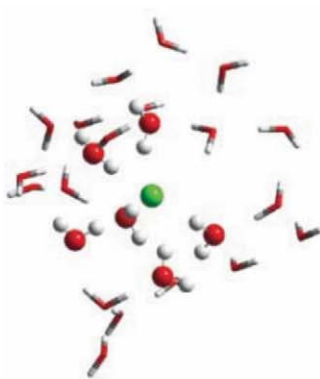


Fig. 4. Computed structure of a 24-water molecule cluster around the Cl^- ion. The outer 18 molecules (stick structure) were frozen and the inner solvation shell structure optimized, and the vibrational spectra were then calculated.

time constant is thus associated with fluctuation in the $\text{OH}\cdots\text{X}^-$ structure. This relaxation time should not be identified with the H-bond lifetime, which is a few picoseconds (11, 12); the measurements reported here cannot separate homogeneous and inhomogeneous contributions to the line width. However, our data are consistent with the ultrafast component in spectral diffusion of the OD oscillator arising from fluctuations in the $\text{OH}\cdots\text{X}^-$ H-bond.

Steady-state and transient IR measurements of the frequency, vibrational lifetime, and orientational relaxation of the OD stretch have been reported as a function of alkali halide concentration; orientational and vibrational relaxation times become longer with increasing concentration, and the vibration shifts to higher frequency (7, 9). In Fig. 3, the frequency and damping constant of the $\text{OH}\cdots\text{Cl}^-$ H-bond mode are plotted as a function of the salt concentration. The frequency shifts to a higher value as the concentration increases, whereas τ_0 is approximately independent of concentration. At the highest ion concentrations (6 M), there are as few as eight water molecules per ion, so most water molecules are involved in the first solvation shell of an ion [the average coordination number for the Cl^- ion, for example, is ~ 6 (5, 14)] and will thus be H-bonded to a water molecule in the solvation shell of an adjacent ion. The blue shift in the $\text{OH}\cdots\text{Cl}^-$ mode at these high concentrations may thus reflect stronger H-bonding as the water molecules become involved in a network of Cl^- and Na^+ ions.

Finally, we address the question of why the anion gives rise to a single low-frequency mode specifically in the isotropic (polarized) Raman spectrum. Although the low-frequency modes of liquid water are certainly delocalized over several molecules, their assignment has often been aided by analogy with clusters of a few molecules (22, 26). For example, in liquid water much of the low-frequency vibrational spectrum can be assigned from a consideration of a cluster of four water molecules H-bonded to a central molecule in a tetrahedral arrangement (22). We

performed density functional theory (DFT) calculations of the Raman spectrum of water clusters around a chloride anion, $\text{Cl}^-(\text{H}_2\text{O})_n$, searching specifically for polarized modes, which could contribute to $R_{\text{iso}}(t)$ and therefore to the spectra reported in Figs. 1 and 2. The $\text{Cl}^-(\text{H}_2\text{O})$ complex yields an H-bond stretching mode calculated at 184 cm^{-1} with $\rho = 0.4$. The frequency is higher than observed experimentally, and there is much evidence from both experiment and calculation that the coordination number for Cl^- is between 5 and 6 (5, 14, 27). Higher clusters produce a number of low-frequency modes, associated with H-bond stretching and bending (figs. S8 and S9), but the vast majority are largely depolarized (ρ greater than 0.7) and will not contribute strongly to the isotropic response. The $\text{Cl}^-(\text{H}_2\text{O})_4$ in a square pyramidal structure with Cl^- at the apex reveals a strongly polarized ($\rho = 0.15$) symmetric stretching mode at 129 cm^{-1} . This result is particularly important in the light of the Car-Parrinello molecular dynamics simulations of Raugei and Klein (13), who reported a basic square pyramidal structure for the first solvation shell of Br^- , supplemented by 1 to 3 water molecules, depending on the coordination number. Addition of a fifth water to the cluster yields a symmetric stretching mode at 147 cm^{-1} with $\rho = 0.24$. The present experimental data are thus consistent with the picture of asymmetric ion solvation that arises in a number of recent theoretical calculations (13, 14, 28). To obtain a more realistic simulation of the spectrum, DFT calculations were performed on the chloride ion solvated by 24 water molecules, based on the tetrahedral structure given by Raugei and Klein; the final structure is shown in Fig. 4. The calculated polarized Raman spectrum reveals a cluster of polarized (ρ below 0.4) modes between 140 cm^{-1} and 185 cm^{-1} , consistent with the experimental observations (Figs. 1 and 2); of course, in the liquid, a broad distribution of structures will exist, but the present observations of the hydrogen bonding vibration are consistent with asymmetric solvation of halides reported in simulations.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/327/5967/857/DC1

Materials and Methods

Figs. S1 to S9

References

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Sea-Level Highstand 81,000 Years Ago in Mallorca

Jeffrey A. Dorale,^{1*} Bogdan P. Onac,^{2*} Joan J. Fornós,³ Joaquin Ginés,³ Angel Ginés,³ Paola Tuccimei,⁴ David W. Peate¹

Global sea level and Earth's climate are closely linked. Using speleothem encrustations from coastal caves on the island of Mallorca, we determined that western Mediterranean relative sea level was ~1 meter above modern sea level ~81,000 years ago during marine isotope stage (MIS) 5a. Although our findings seemingly conflict with the eustatic sea-level curve of far-field sites, they corroborate an alternative view that MIS 5a was at least as ice-free as the present, and they challenge the prevailing view of MIS 5 sea-level history and certain facets of ice-age theory.

Large fluctuations in global sea level occurred throughout the last interglacial/glacial cycle, but the precise magnitudes of some of these fluctuations are subjects of energetic debate. The eustatic (ice equivalent) sea-level height of the marine isotope stage (MIS) 5a highstand event is among the more controversial of these sea-level variations, with estimates ranging widely from +3 to -30 m relative to modern sea level (1–6). Along the coast of Mallorca in the western Mediterranean (Fig. 1A), caves exist that provide an extraordinary setting for capturing past sea-level changes. The caves formed by the mixing of fresh water and seawater in the coastal phreatic zone (7) and contain numerous speleothems (such as stalactites and stalagmites) that formed in early Quaternary time when the caves were air-filled chambers. Throughout the Middle and Late Quaternary, the caves were repeatedly flooded by glacioeustatic sea-level oscillations. The water level of each flooding event was recorded by a distinct encrustation of calcite or aragonite over existing speleothems and along cave walls (Fig. 1B). Similar encrustations form

at present in a low-amplitude tide-controlled microenvironment, at or a few centimeters above and below the water table (Fig. 1, C, D, and G). U/Th dating of the outside of these modern encrustations yields present-day ages, whereas subsamples from their interiors yield ages of ~2.8 thousand years ago (ka) (8), demonstrating they are indeed “modern” and also that mean sea level has remained stable on Mallorca for the past ~2800 years. We have identified several well-defined encrustation levels below and above the present-day sea level corresponding to older sea-level stands (Fig. 1, E and F), and we used such encrustations to determine relative sea level during MIS 5a.

We collected six speleothem encrustations from five different caves along the eastern and southern coast of Mallorca (Fig. 1A). All the sampled caves in our study are within a horizontal distance of 250 m of the coast; thus, the water table of the caves is, and was in the past, coincident with sea level. The encrustations were sampled from two distinct horizons located at 1.2 to 1.5 and 2.6 m (±5 cm) above present sea level. The errors associated with sample elevations are well within the 50-cm maximum amplitude of water-level oscillations due to barometric tides. Two subsamples were dated from most overgrowths (9); the main subsample was extracted from the widest part of the overgrowth, thought to represent the mean sea-level position. When possible, another subsample was milled within 2 to 8 cm above the main one.

Three stages of sea-level encrustations were identified by U/Th ages of 80 to 82 ka, 116 ka, and 121 ka (Table 1). The ages of 116 ka and 121 ka obtained for two encrusted speleothems collected from elevations of ~2.6 m are consistent

with previous results for MIS 5e sea level in Mallorca (1, 10) and are also consistent, within a few meters, with many other estimates from around the world of eustatic sea level during MIS 5e. Although we cannot be certain that peak MIS 5e sea level has been captured by our sampled encrustations at 2.6 m, the observation nonetheless suggests that tectonic motion is not a major confounding factor in our reconstruction of MIS 5a sea level, because it is implausible that MIS 5a deposits could be significantly elevated by tectonics while MIS 5e deposits were not. Our results at face value therefore indicate that between ~82 and 80 ka, sea level in Mallorca stood at ~+1 m relative to present sea level (Table 1 and Fig. 2A).

If interpreted solely as a change in ice-equivalent sea level, our ~+1 m MIS 5a highstand conflicts with reconstructions from raised coral reefs from uplifting coastlines (such as Haiti, Barbados, and New Guinea), which suggest that MIS 5a eustatic sea level was anywhere from 7 m (one “Greenland equivalent”) to 30 m (four “Greenland equivalents”) below present sea level (2, 3, 11, 12). Lower-than-present sea levels at ~80 ka have also been inferred from submerged speleothems from Grand Bahama Island (13) and coral reefs on the Florida Keys (14).

Relative sea-level change at a site, however, reflects not only changes in global ice volume but also the response of Earth to changes in surface loading in the form of surface deformation and geoid changes, or glacial isostatic adjustment (GIA) (3, 15). Thus, at any site, and in the absence of tectonic effects, relative sea level in its most simple expression reflects two unknown parameters: GIA and eustasy. GIA has both glacio- and hydro-components (3, 15, 16). The Mediterranean Sea is an intermediate-field basin, moderately distant from the former major glaciation centers of the Northern Hemisphere (16). Thus, the ~1-m elevation of the ~81-ka highstand at Mallorca may plausibly contain a significant effect of GIA associated with Northern Hemisphere ice sheet history. A similar scenario has been proposed for other intermediate-field sites that display evidence for a near-modern MIS 5a sea level (5), so as to remain consistent with the far-field estimates of MIS 5a eustatic sea level from Barbados and New Guinea of ~-10 to -20 m.

Numerical models generally estimate GIA with assumptions about the thickness, distribu-

¹Department of Geoscience, University of Iowa, 121 Trowbridge Hall, Iowa City, IA 52242, USA. ²Department of Geology, University of South Florida, 4202 East Fowler Avenue, SCA 528, Tampa, FL 33620, USA; and Department of Geology, Babes-Bolyai University, Emil Racovita Institute of Speleology Cluj, Romania. ³Departament de Ciències de la Terra, Universitat de les Illes Balears, Carretera Valldemossa km 7.5, Palma de Mallorca, 07122, Spain. ⁴Dipartimento di Scienze Geologiche, Università di Roma III, Largo St. Leonardo Murialdo, 1, 00146 Roma, Italy.

*To whom correspondence should be addressed. E-mail: jeffrey-dorale@uiowa.edu (J.A.D.); bonac@cas.usf.edu (B.P.O.). These authors contributed equally to this work.

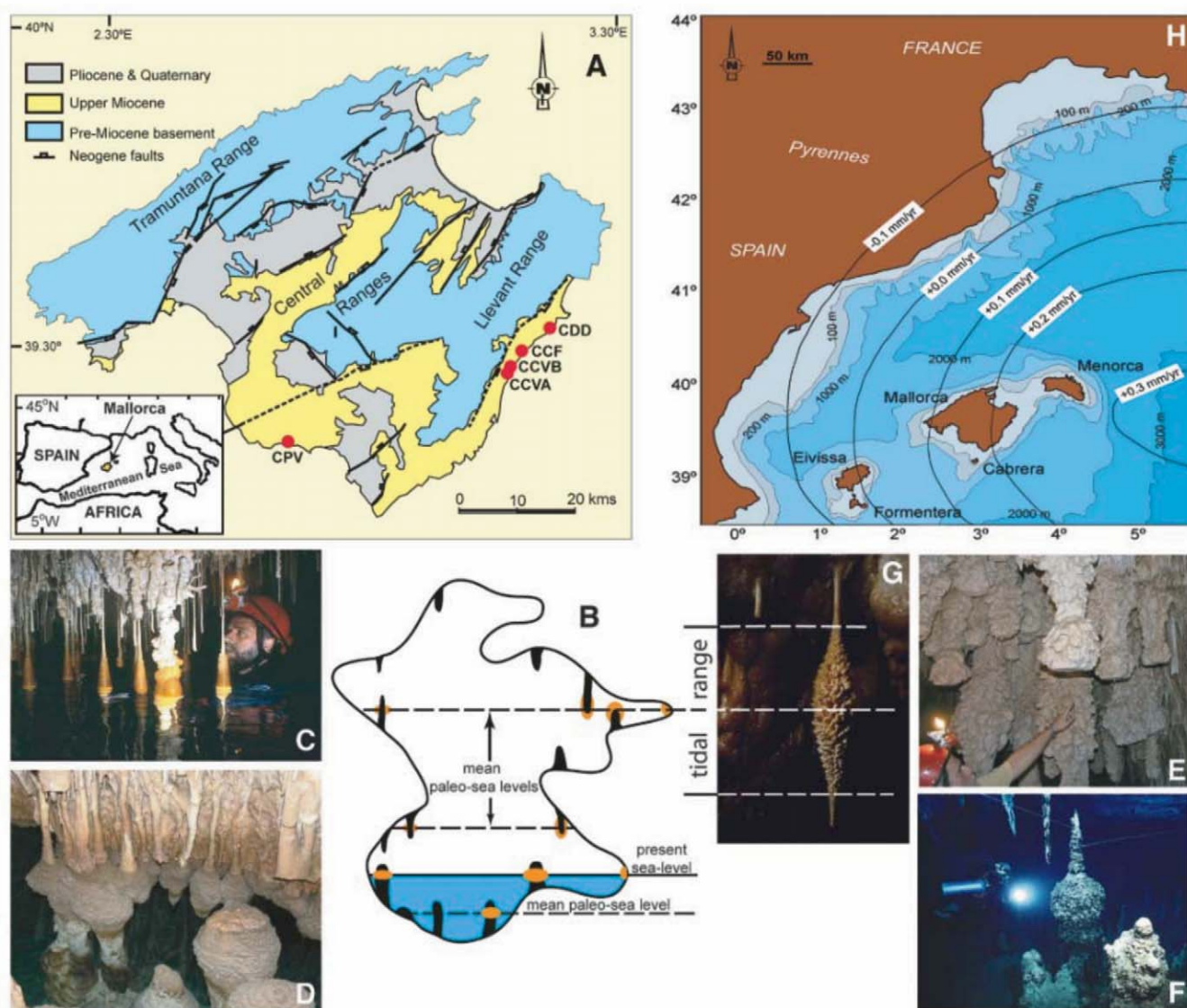


Fig. 1. Encrusted speleothems at various levels in caves from Mallorca. (A) Geologic map of Mallorca (10) and location of sampled caves (red dots). (B) Schematic cross-section through a coastal cave in Mallorca showing multiple carbonate encrustation levels. (C and D) Present-day and paleo levels of encrusted

speleothems related to higher (E) and lower (F) sea-level stands. (G) Typical morphology for tidal range-related carbonate encrustation (size of speleothem, 20 cm). (H) Bathymetric map of the western Mediterranean region and the predicted present-day rate of sea-level change due to GIA [adapted from (15)].

tion, and duration of former ice loads as well as the viscoelastic properties of Earth. Because eustatic sea level has been relatively stable during the late Holocene (17), this is a useful period for comparing the predictions of GIA models and the field evidence of sea-level changes. Some models predict a nearly constant sea-level rise on the order of 0.2 mm/year in the vicinity of Mallorca throughout the late Holocene, due to hydroisostatic subsidence of the basin (15, 18) (Fig. 1H). This equates to 60 cm of sea-level rise over the past 3000 years. But the modern, actively accreting speleothem encrustations from Mallorca (Fig. 1, C and D) show instead that relative sea level has remained stable over the past ~2800 years (8). We therefore propose that Mallorca's location in the western Mediterranean may actually

be somewhat unusual with regard to GIA. According to the models of (15) and (18), Mallorca is fairly close to (within 100 km) the predicted zone of neutral GIA to the west (Fig. 1H). Mallorca is also part of a shallow-water shelf extending from the Iberian mainland (Fig. 1H) that would probably have some effect on the hydroisostatic properties of the site, but this detail has probably not been incorporated into GIA models because of their relatively coarse resolution. In any case, the late Holocene field evidence from Mallorca does indeed indicate that the GIA effects of (15) and (18) have been overestimated for this region, suggesting the possibility that Mallorca occupies a narrow transition zone between regions of emergence and submergence in the Mediterranean basin, where sea level nearly follows the eustatic curve (19).

From our data and that of (10) and (20), we estimate that the MIS 5a sea-level highstand involved very rapid ice melting leading up to the event and had a duration on the order of 4000 years, from ~84 to 80 ka (Fig. 2C). This duration is similar to estimates from Bermuda of ~5000 years (2). Specifically at Mallorca, speleothem encrustations record a MIS 5b sea-level height of ~-20 m at 85.4 ± 0.9 ka and a MIS 5a height of ~+1 m by 84.2 ± 1.0 ka (10). The sea-level drop after the MIS 5a highstand was very rapid as well, because speleothem encrustations record a height of ~-15 m by ~78.6 $\pm$ 0.8 ka (10). These rates of sea-level change nominally approach 20 m per thousand years (ky), which is comparable to the meltwater pulses of the last major deglaciation (21) and almost 30 times

larger than the largest observed or predicted rates of GIA (15). Thus, the MIS 5b/5a/4 sequence at Mallorca demonstrates that very large eustatic changes were involved with the MIS 5a highstand. We therefore consider the simple interpretation of our data that eustatic sea level during MIS 5a stood around +1 m relative to present sea level, implying less ice on Earth 81,000 years ago than today. Although this interpretation conflicts with the generally accepted eustatic sea-level curve

based on the far-field sites of Barbados and New Guinea, it is consistent with a number of other estimates from around the world, including those from the Bahamas, the U.S. Atlantic Coastal Plain, Bermuda, Cayman Islands, and California (4, 6, 22–26) (Fig. 2B). We considered the simple fact that this geographically diverse suite of sites spans a wide range of presumed isostatic states, yet the suite consistently indicates a late MIS 5a highstand of ~+0 to 3 m (Fig. 2B).

Bermuda and Mallorca, for example, are both tectonically stable, and both have MIS 5e/5a estimates of 2 to 3 and 1 to 2 m above modern sea level, respectively; whereas MIS 5e/5a estimates from Barbados are ~+5 m and ~−18 m (2). Any appeal to GIA to account for these discrepancies must somehow take into account the unlikely outcome that different ice centers on different continents (Laurentide versus Fennoscandian) would generate the virtually

Table 1. Sample information and U/Th data. Errors are 2σ of the mean and are based on the analytical precision. m apsl, meters above present sea level; ppm, parts per million; AR, activity ratio.

Cave	Sample code	Elevation (m apsl)	U (ppm)	²³⁴ U/ ²³⁸ U (initial)	²³⁴ U/ ²³⁸ U (measured)	²³⁰ Th/ ²³² Th AR	²³⁰ Th/ ²³⁴ U AR	Age (ka) ±2σ
Cova de Cala	CCVA-1	1.25	0.110 ± 0.022	1.476 ± 0.086	1.377 ± 0.069	9077	0.5450 ± 0.0021	81.95 ±0.56
Varques A	CCVA-2	1.30	0.121 ± 0.018	1.478 ± 0.069	1.379 ± 0.055	5599	0.5440 ± 0.0020	81.66 ±0.45
Cova del Dimoni	CDD-1	1.48	1.191 ± 0.019	1.223 ± 0.038	1.178 ± 0.003	18814	0.5297 ± 0.0039	80.44 ±0.61
	CDD-2	1.45	1.186 ± 0.018	1.222 ± 0.034	1.177 ± 0.027	17160	0.5298 ± 0.0028	80.65 ±0.46
Cova de Cala	CCVB	1.32	0.101 ± 0.021	1.473 ± 0.021	1.376 ± 0.017	418	0.5413 ± 0.0046	80.78 ±0.96
Varques B								
Cova de Cala Falcó	CCF-1	1.57	0.208 ± 0.023	2.397 ± 0.011	2.113 ± 0.009	2410	0.5510 ± 0.0023	80.43 ±0.48
	CCF-2	1.53	0.202 ± 0.021	2.431 ± 0.015	2.137 ± 0.012	990	0.5563 ± 0.0023	81.10 ±0.49
Cova des Pas de Vallgornera	CPV-1	1.60	0.156 ± 0.030	1.408 ± 0.024	1.325 ± 0.019	31537	0.5350 ± 0.0028	80.09 ±0.48
	CPV-2	1.52	0.144 ± 0.028	1.413 ± 0.026	1.329 ± 0.021	34757	0.5361 ± 0.0019	80.12 ±0.45
	CPV-B8	1.52	0.119 ± 0.018	1.492 ± 0.020	1.391 ± 0.016	1812	0.5410 ± 0.0022	80.97 ±0.48
	CPV-B6	2.60	0.108 ± 0.020	1.198 ± 0.018	1.141 ± 0.013	1892	0.6830 ± 0.0027	120.60 ±0.89
	CPV-B9	2.60	0.122 ± 0.014	1.240 ± 0.017	1.173 ± 0.012	1151	0.6710 ± 0.0019	116.20 ±0.61

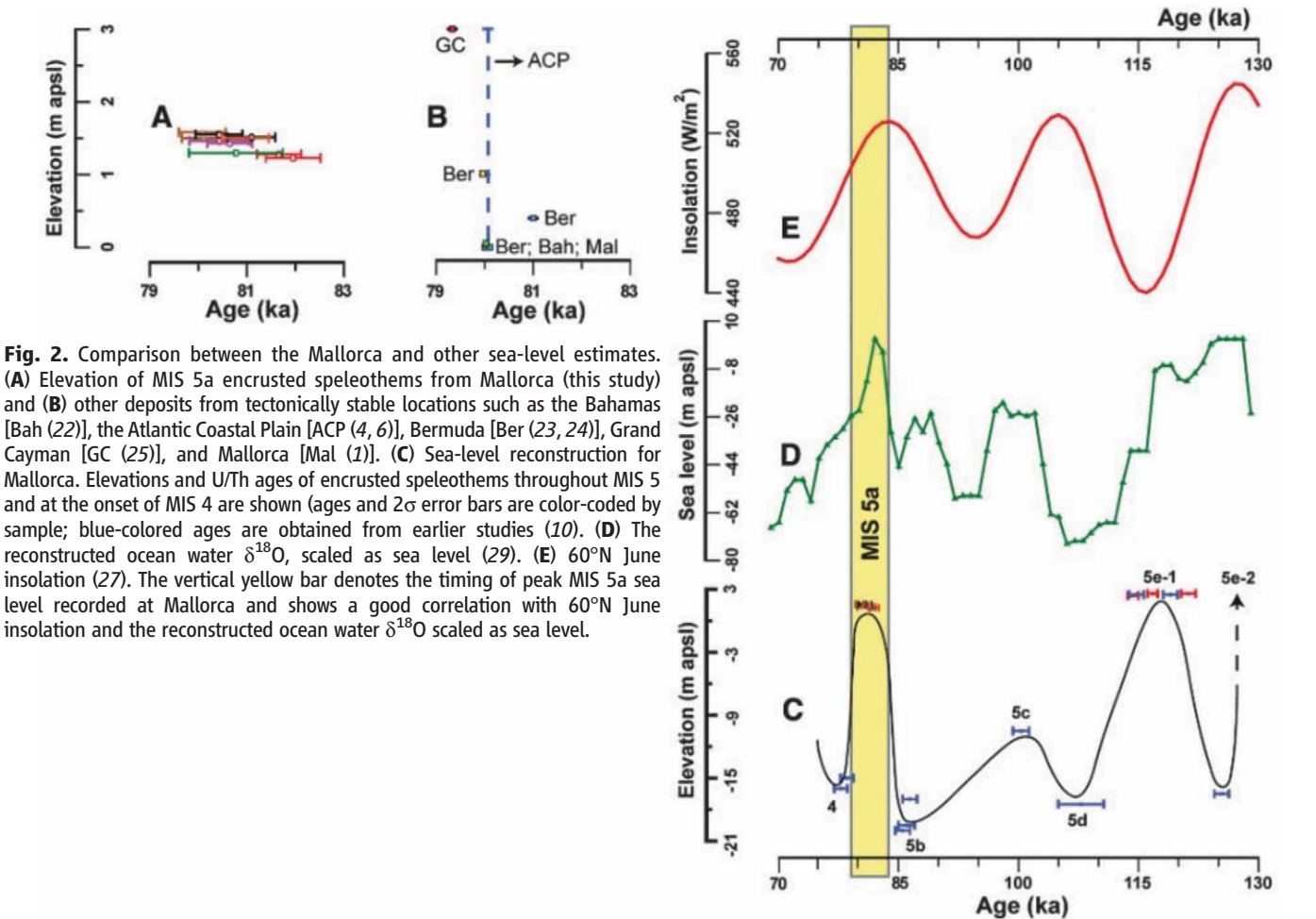


Fig. 2. Comparison between the Mallorca and other sea-level estimates. (A) Elevation of MIS 5a encrusted speleothems from Mallorca (this study) and (B) other deposits from tectonically stable locations such as the Bahamas [Bah (22)], the Atlantic Coastal Plain [ACP (4, 6)], Bermuda [Ber (23, 24)], Grand Cayman [GC (25)], and Mallorca [Mal (1)]. (C) Sea-level reconstruction for Mallorca. Elevations and U/Th ages of encrusted speleothems throughout MIS 5 and at the onset of MIS 4 are shown (ages and 2σ error bars are color-coded by sample; blue-colored ages are obtained from earlier studies (10)). (D) The reconstructed ocean water δ¹⁸O, scaled as sea level (29). (E) 60°N June insolation (27). The vertical yellow bar denotes the timing of peak MIS 5a sea level recorded at Mallorca and shows a good correlation with 60°N June insolation and the reconstructed ocean water δ¹⁸O scaled as sea level.

identical MIS 5e/5a relative sea-level histories of tectonically stable Bermuda and Mallorca. The very rapid onset and relatively brief nature of the MIS 5a highstand may have plausibly generated lags between the timing of sea-level changes and the timing of coral reef growth, and may provide a partial explanation as to why reefs on Barbados and New Guinea do not record a comparable eustatic height for this event. This and other factors that could be part of the apparent discrepancy are discussed in (9).

The suggestion that MIS 5a sea level was slightly higher than at present and only slightly lower than the MIS 5e sea level implies that most of the ice built up during MIS 5b would have melted during the onset of MIS 5a. The ~84- to 80-ka timing of this highstand closely matches the June 60°N insolation peak at ~84 ka (Fig. 2E), a pattern that is consistent with the Milankovitch model. In fact, June insolation at 60°N was higher at ~84 ka than that at 11 ka (27), and field studies in the Baffin Island region suggest the complete melting of the Laurentide Ice Sheet around 80 ka (28). Finally, we find additional, independent support for a near-modern eustatic MIS 5a highstand when we consider the indirect sea-level estimate of (29) inferred from a Pacific benthic $\delta^{18}\text{O}$ record, the Vostok atmospheric $\delta^{18}\text{O}$ record, and certain assumptions about the Dole effect on deep-water temperatures (Fig. 2D). The premise of the approach of (29) is that the deep-sea $\delta^{18}\text{O}$ record does not capture the true magnitude of eustatic sea-level change, because the $\delta^{18}\text{O}$ signal is partially controlled by temperature.

Because of its relation to continental ice volume, an accurate Quaternary sea-level curve has

been a long-term goal of scientists interested in ice-age cycles and their causes. Ice-age theory has long assumed gradual ice buildup and more rapid ice melting in the generally accepted model of the ~100-ky cycle of glaciation. Instead, the emerging body of evidence suggests that both melting and accumulation can be very rapid during discrete intervals of time when specific conditions prevail. Furthermore, the 100-ky model of glaciation has always faced the problem that although the deep-sea $\delta^{18}\text{O}$ record is dominated by a 100-ky cycle, northern high-latitude summer insolation has negligible power in this band. Our data from Mallorca and data from other sites around the world indicate the possibility that eustatic sea level was near modern levels at ~80 ka. If this is true, the 100-ky cycle so universally accepted as the main rhythm of the Middle and Late Quaternary glaciations, in fact, applies rather poorly to ice growth and decay, but much better to carbon dioxide, methane, and temperatures recorded by polar ice (30).

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A Genetic Variant BDNF Polymorphism Alters Extinction Learning in Both Mouse and Human

Fatima Soliman,^{1,2*} Charles E. Glatt,² Kevin G. Bath,² Liat Levita,^{1,2} Rebecca M. Jones,^{1,2} Siobhan S. Pattwell,² Deqiang Jing,² Nim Tottenham,^{1,2} Dima Amso,^{1,2} Leah H. Somerville,^{1,2} Henning U. Voss,³ Gary Glover,⁴ Douglas J. Ballon,³ Conor Liston,^{1,2} Theresa Teslovich,^{1,2} Tracey Van Kempen,^{1,2} Francis S. Lee,^{2*} B. J. Casey^{1,2*}

Mouse models are useful for studying genes involved in behavior, but whether they are relevant to human behavior is unclear. Here, we identified parallel phenotypes in mice and humans resulting from a common single-nucleotide polymorphism in the brain-derived neurotrophic factor (BDNF) gene, which is involved in anxiety-related behavior. An inbred genetic knock-in mouse strain expressing the variant BDNF recapitulated the phenotypic effects of the human polymorphism. Both were impaired in extinguishing a conditioned fear response, which was paralleled by atypical frontoamygdala activity in humans. Thus, this variant BDNF allele may play a role in anxiety disorders showing impaired learning of cues that signal safety versus threat and in the efficacy of treatments that rely on extinction mechanisms, such as exposure therapy.

Genetically modified mice provide useful model systems for testing the role of candidate genes in behavior. The extent to

which such genetic manipulations in the mouse and the resulting phenotype can be translated across species, from mouse to human, is less clear. In this

report, we focused on identifying biologically valid phenotypes across species. We utilized a common single-nucleotide polymorphism (SNP) in the brain-derived neurotrophic factor (BDNF) gene that leads to a valine (Val) to methionine (Met) substitution at codon 66 (Val66Met). In an inbred genetic knock-in mouse strain that expresses the variant BDNF allele to recapitulate the specific phenotypic properties of the human polymorphism in vivo, we found the BDNF Val66Met genotype was associated with treatment-resistant forms of anxiety-like behavior (1). The objective of this study was to test if the Val66Met genotype could affect extinction learning in our mouse model and whether such findings could be generalized to human populations.

¹The Sackler Institute for Developmental Psychobiology, Weill Cornell Medical College, New York, NY 10065, USA.

²Department of Psychiatry, Weill Cornell Medical College, New York, NY 10065, USA. ³Citigroup Biomedical Imaging Center, Department of Radiology, Weill Cornell Medical College, New York, NY 10065, USA. ⁴Lucas Center for Imaging, Department of Radiology, Stanford University, Stanford, CA 94305, USA.

*To whom correspondence should be addressed. E-mail: fas2002@med.cornell.edu (F.S.) or fslee@med.cornell.edu (F.S.L.) or bjcc2002@med.cornell.edu (B.J.C.)

BDNF mediates synaptic plasticity associated with learning and memory (2, 3), specifically in fear learning and extinction (4, 5). BDNF-dependent forms of fear learning have known biological substrates and lie at the core of a number of clinical disorders (6, 7) associated with the variant BDNF (8–10). Fear-learning paradigms require the ability to recognize and remember cues that signal safety or threat and to extinguish these associations when they no longer exist. These abilities are impaired in anxiety disorders such as posttraumatic stress disorder and phobias (11, 12). Behavioral treatments for these disorders such as exposure therapy rely on basic principles of extinction learning (13) in which an individual is repeatedly exposed to an event that was previously associated with aversive consequences. Understanding the effect of the BDNF Met allele on these forms of learning can provide insight into the mechanism of risk for anxiety disorders, can refine existing treatments, and may lead to genotype-based personalized medicine.

We examined the impact of the variant BDNF on classic fear conditioning and extinction paradigms that were adapted to be suitable for each species and that are associated with well-known underlying biological substrates (14, 15, 16). Fear conditioning consisted of pairing a neutral cue with an aversive stimulus. With repeated pairings, the cue itself takes on properties of the aversive stimulus as it predicts threat of an impending aversive event. Extinction consisted of presenting the cue alone following conditioning, whereby the association is diminished with repeated exposure to empty threat.

We tested 68 mice (17 BDNF^{Val/Val}, 33 BDNF^{Val/Met} and 18 BDNF^{Met/Met}) and 72 humans group-matched for age, gender, and ethnic background (36 Met allele carriers: 31 Val/Met and 5 Met/Met, and 36 non-Met allele carriers: Val/Val) (table S1). We found no effect of the BDNF Met allele on fear conditioning in

mice as measured by the percentage of time spent freezing in response to the conditioned stimulus ($F_{2,65} = 1.58$, $P < 0.22$) (fig. S1A) or on general fear arousal as measured by freezing during the intertrial interval (fig. S2). We grouped human Met allele carriers together (Val/Met and Met/Met) for analyses, because the rarity of human Met allele homozygotes prevents enough observations for meaningful analysis. As we found in the mouse, there was no effect of the BDNF Met allele on fear conditioning in humans as measured by skin conductance response to the cue predicting the aversive stimulus relative to a neutral cue ($F_{1,70} = 0.67$, $P < 0.42$) (fig. S1B).

Analysis of extinction trials showed a main effect of genotype for both mice [$(F_{2,65} = 6.55$, $P < 0.003)$; Val/Val, 48.8 ± 2.3 ; Val/Met, 53.2 ± 1.8 ; Met/Met, 61.3 ± 2.8] and humans [$(F_{1,70} = 4.86$, $P < 0.03)$; Val/Val, 0.32 ± 0.03 ; Val/Met, 0.42 ± 0.04], such that extinction learning was impaired in Met allele carriers relative to non-Met allele carriers. The Met allele carriers showed slower extinguishing, as indicated by an interaction of time $\times$ genotype for the mouse ($F_{2,65} = 6.51$, $P < 0.003$) with no differences in freezing initially, but a dose response of the Met allele on the percentage of freezing behavior during late trials [Val/Val versus Val/Met: $t(48) = -2.62$, $P < 0.01$; Val/Val versus Met/Met: $t(33) = -4.78$, $P < 0.0001$; Val/Met versus Met/Met: $t(49) = -2.90$, $P < 0.006$] (Fig. 1A). Humans showed a similar pattern to the mice with no genotypic difference in the initial human skin conductance response during early trials of extinction [$t(70) = -1.57$, $P < 0.12$], but significant differences by late trials [$t(70) = -2.43$, $P < 0.02$, corrected for time] (Fig. 1B). These data demonstrate slower or impaired extinction related to the Met allele in both mouse and human.

The learning paradigm for humans included a conditioned stimulus paired with the aversive stimulus and a neutral stimulus that was not paired with the aversive stimulus. This design

allowed for distinguishing between effects due to impaired learning versus a general effect of heightened anxiety, as generalized heightened anxiety would lead to a similar response to both the conditioned and neutral cues. Met allele carriers had an overall heightened response to both conditioned and neutral cues [main effect of genotype ($F_{1,70} = 7.21$, $P < 0.009$)], but overall, they differentiated between the conditioned and neutral cues similarly to the non-Met allele carriers (fig. S1B). Yet, when we examined these effects over time, Met allele carriers took longer to recognize that the neutral cue was not associated with the aversive stimulus, as evidenced by significant genotypic differences during late trials [$t(70) = -3.46$, $P < 0.001$, corrected for time] but not early trials [$t(70) = -1.44$, $P < 0.16$] (Fig. 2). Thus, the skin conductance response to the neutral cue during fear conditioning showed a pattern similar to that observed during extinction trials (16).

The genetic findings for both fear conditioning and extinction suggest that learning about cues that signal threat of an impending aversive event is intact in Met allele carriers. However, learning that cues no longer signal threat (e.g., extinction) or do not predict threat (cues not paired with an aversive stimulus) is impaired in Met allele carriers, which leads to exaggerated and longer retention of aversive responses where they are not warranted.

To provide neuroanatomical evidence to validate our cross-species translation, we used human functional magnetic resonance imaging (MRI) to define the underlying neural circuitry of the behavioral effects of BDNF Val66Met and to map them to known circuits involved in fear learning in the rodent (table S2). We targeted frontoamygdala circuitry that has been demon-

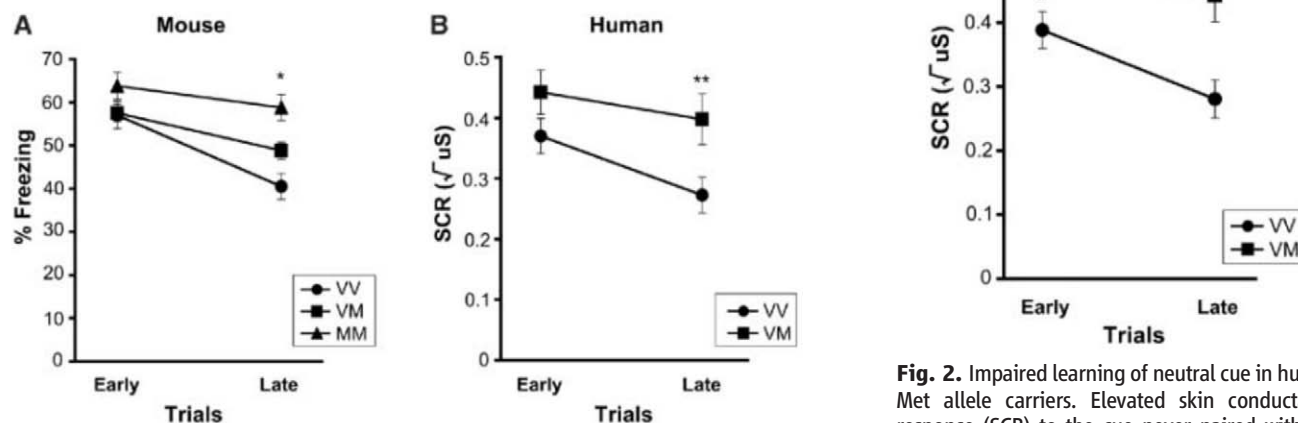


Fig. 1. Altered extinction in mice and humans with BDNF Val66Met. Impaired extinction in Met allele carriers (Val/Met and Met/Met) as a function of time in 68 mice (A) and 72 humans (B) as indexed by percentage of time freezing in mice and skin conductance response (SCR) in humans to the conditioned stimulus when it was no longer paired with the aversive stimulus. All results are presented as means $\pm$ SEM. * $P < 0.01$, Student's t test. ** $P < 0.02$, Student's t test. VV, Val/Val; VM, Val/Met; and MM, Met/Met.

Fig. 2. Impaired learning of neutral cue in human Met allele carriers. Elevated skin conductance response (SCR) to the cue never paired with the aversive stimulus during fear conditioning as a function of time in Met allele carriers (VM) relative to non-Met allele carriers (VV). All results are presented as means $\pm$ SEM. * $P < 0.001$, Student's t test. VV, Val/Val; VM, Val/Met.

strated to support fear conditioning and extinction in both rodent (17–20) and human (21–26) studies. Whereas portions of the amygdala have been shown to be essential for fear conditioning (27, 28), ventral prefrontal cortical regions have been shown to be important for modifying previously learned associations and extinction (19, 29). Thus, on the basis of our behavioral findings in the mouse and human, we hypothesized that ventromedial prefrontal regions, important in extinction, would be less active in Met allele carriers relative to non-Met allele carriers and that amygdala activity may be enhanced.

To test this hypothesis, we examined the main effect of genotype on brain activity during extinction of the previously conditioned stimulus. The analysis directly parallels the observed behavioral main effects of genotype on extinction as measured by mean percentage of time freezing in the mice (Fig. 3A) and mean skin conductance response in humans (Fig. 3B) (16) with Met allele carriers showing weaker extinction. The imaging results showed significantly less ventromedial prefrontal cortical (vmPFC) activity during extinction in Met allele carriers relative to non-Met allele carriers [$t(68) = -3.78$, $P < 0.05$, corrected] (Fig. 3C) (16). In contrast, Met allele carriers show greater amygdala activity relative to non-Met allele carriers during extinction [$t(68) = 2.23$, $P < 0.05$, corrected] (Fig. 3D). These findings indicate that cortical regions previously shown to be essential for extinction (vmPFC) in both rodent and human (19, 26, 30) are hyporesponsive in Met allele carriers relative to non-Met allele carriers. Moreover, Met allele carriers show continued recruitment of the amygdala, a region that should show diminished activity during the extinction trials of the experiment (26). These findings are most likely due to the SNP biasing activity-dependent learning

rather than affecting CNS development per se, as there was no evidence of genotypic developmental effects on brain structure in this ethnicity-, age-, and gender-matched sample from MRI-based brain morphometry (supporting online text). Furthermore, an association between vmPFC activity and the strength of fibers connecting frontolimbic regions is consistent with more effective extinction learning as a result of better vmPFC modulation of the amygdala (supporting online text, figs. S3 and S4).

These experiments identify a behavioral phenotype related to BDNF Val66Met across species providing evidence for translation from mouse to human. The mouse model provides the opportunity to test dose-dependent effects of the BDNF Met allele in both a controlled genetic and environmental background not feasible in humans. These features allow for reliable assignment of behavioral differences to the effects of the Val66Met polymorphism. The human behavioral and imaging findings provide confidence that cross-species translation is biologically valid, by defining the underlying neural circuitry of the behavioral effects of BDNF Val66Met that can be mapped onto known circuits involved in fear learning and extinction. The robustness of our findings across species and paradigms is evidenced by work showing slower extinction coupled with decreased neuronal dendritic complexity in vmPFC in the BDNF^{Met/Met} mice in a conditioned taste aversion task compared with wild-type counterparts (31). Furthermore BDNF^{Met/Met} mice exhibit a trend toward blunted expression of c-Fos in the vmPFC as compared with wild-type mice after the fear extinction paradigm (supporting online text and fig. S5).

Impaired extinction learning has been implicated in anxiety disorders, including phobias and posttraumatic stress disorder, whereby the indi-

vidual has difficulty recognizing an event as safe (32). Our neuroimaging findings of diminished ventromedial prefrontal activity and elevated amygdala activity during extinction are reminiscent of those reported in patients with anxiety disorders and depression when presented with an empty threat or aversive stimuli (e.g., fearful faces) (33, 34). Understanding the effect of the BDNF Met allele on specific components of a simple form of learning provides insight into risk for anxiety disorders and has important implications for the efficacy of treatments for these disorders that rely on extinction mechanisms. One such treatment is exposure therapy, whereby an individual is repeatedly exposed to a traumatic event in order to diminish the significance of that event. Our findings suggest that the BDNF Val66Met SNP may play a key role in the efficacy of such treatments and may ultimately guide personalized medicine for related clinical disorders.

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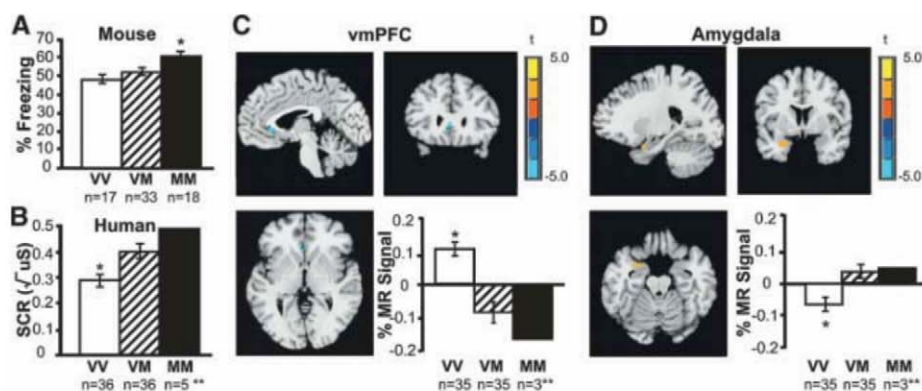


Fig. 3. Neural circuitry of the behavioral effect of BDNF Val66Met during extinction. **(A)** Average percentage of time freezing during extinction by genotype in 68 mice. **(B)** Average skin conductance response (SCR) during extinction by genotype in 72 humans. **(C)** Brain activity as indexed by percent change in magnetic resonance (MR) signal during extinction in the ventromedial prefrontal cortex (vmPFC) by genotype ($x, y, z = -4, 24, 3$), with Met allele carriers having significantly less activity than Val/Val homozygotes ($VM < VV$ is blue), image threshold $P < 0.05$, corrected. **(D)** Genotypic differences in left amygdala activity during extinction ($x, y, z = -25, 2, -20$) in 70 humans, with Met allele carriers having significantly greater activity than Val/Val homozygotes ($VM > VV$ is orange), image threshold $P < 0.05$, corrected. * $P < 0.05$. **MM were included in the analysis with VM, but plotted separately to see the dose response. All results are presented as means $\pm$ SEM. VV, Val/Val; VM, Val/Met; MM, Met/Met.

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Figs. S1 to S5
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Vibrio cholerae VpsT Regulates Matrix Production and Motility by Directly Sensing Cyclic di-GMP

Petya V. Krasteva,¹ Jiunn C. N. Fong,² Nicholas J. Shikuma,² Sinem Beyhan,²
Marcos V. A. S. Navarro,¹ Fitnat H. Yildiz,^{2*} Holger Sondermann^{1*}

Microorganisms can switch from a planktonic, free-swimming life-style to a sessile, colonial state, called a biofilm, which confers resistance to environmental stress. Conversion between the motile and biofilm life-styles has been attributed to increased levels of the prokaryotic second messenger cyclic di-guanosine monophosphate (c-di-GMP), yet the signaling mechanisms mediating such a global switch are poorly understood. Here we show that the transcriptional regulator VpsT from *Vibrio cholerae* directly senses c-di-GMP to inversely control extracellular matrix production and motility, which identifies VpsT as a master regulator for biofilm formation. Rather than being regulated by phosphorylation, VpsT undergoes a change in oligomerization on c-di-GMP binding.

In *Vibrio cholerae*, biofilm formation is facilitated by colonial morphotype variation (1–4). Rugose variants produce increased levels of extracellular matrix by means of expression of *Vibrio* polysaccharide (*vps*) genes and genes encoding matrix proteins. *vps* expression is under the control of two positive transcriptional regulators, VpsT and VpsR (5, 6). VpsT is a member of the FixJ, LuxR, and CsgD family of prokaryotic response regulators, typically effectors in two-component signal transduction systems that use phosphoryl transfer from upstream kinases to modulate response-regulator protein activity (7–9). Although the putative phosphorylation site is conserved in VpsT's receiver domain, other residues crucial for phosphotransfer-dependent signaling are not, and no cognate kinase has been identified to date (fig. S1). Regulation by VpsT and VpsR has been linked to signal transduction by using the bacterial second messenger cyclic di-guanosine monophosphate (c-di-GMP) (10, 11) (fig. S2), yet little is known about the direct targets of the nucleotide. A ribo-switch has been identified as a c-di-GMP target that regulates gene expression of a small number of genes, but that is unlikely to account for the global change in transcriptional profile required for biofilm formation (12). Neither do PilZ domain-containing proteins, potential c-di-GMP effectors, affect rugosity, because a *V. cholerae*

strain lacking all five PilZ domain-containing proteins retains its colony morphology and ability to overproduce *vps* gene products (13).

VpsT consists of an N-terminal receiver (REC) domain and a C-terminal helix-turn-helix (HTH) domain, with the latter mediating DNA binding (Fig. 1A) (14) (also see supporting online text for details). Unlike other REC domains, the canonical (α/β)₅-fold in VpsT is extended by an additional helix at its C terminus [helix α_6 in (Fig. 1A)]. The HTH domain buttresses an interface formed by helices 3 and 4 of the N-terminal regulatory domain. There are two nonoverlapping dimerization interfaces between noncrystallographic VpsT protomers [chain A-chain B and chain A-chain B^{sym} (symmetrical) in (Fig. 1B)]. The c-di-GMP-independent interface involves interactions mediated by a methionine residue (M¹⁷) (15) located at the beginning of α_1 and a binding pocket that extends into the putative phosphorylation site of the REC domain (fig. S3A). The second interface involves α_6 of the REC domain, in contrast to canonical response regulators, such as CheY and PhoB, that utilize a surface formed by α_4 - β_5 - α_5 for dimerization (9). The binding of two intercalated c-di-GMP molecules to the base of α_6 stabilizes VpsT dimers using this interface (Fig. 1 and fig. S3B).

The binding motif for c-di-GMP in VpsT consists of a four-residue-long, conserved W[F/L/M][T/S]R sequence (15) (fig. S1). The side chains of the tryptophan and arginine form π -stacking interactions with the purine rings of the nucleotide (Fig. 1C). While the hydrophobic residue in the second position plays a structural role where it is buried in the REC domain, the

threonine residue at position 3 forms a hydrogen bond with the phosphate moiety of c-di-GMP. A subclass of VpsT and/or CsgD homologs exists with a proline substitution in position 3 (W[F/L/M]PR). Although CsgD is also functionally linked to c-di-GMP signaling in *Escherichia coli* and *Salmonella* (16, 17), its binding pocket appears to be distinct from that of VpsT, as it displays a highly conserved YF[T/S]Q motif that is unlikely to accommodate c-di-GMP (fig. S3B).

The apparent affinity of VpsT for c-di-GMP, determined by isothermal titration calorimetry, is 3.2 μ M with 1:1 stoichiometry, consistent with a dimer of c-di-GMP binding to a dimer of VpsT (fig. S4A). Single point mutations in the conserved c-di-GMP-binding motif (VpsT^{R134A}, VpsT^{W131F}, or VpsT^{T133V}) or in the isoleucine in α_6 of the c-di-GMP-stabilized REC dimerization interface (VpsT^{I141E}) abolished c-di-GMP binding, which indicated that dimeric REC domains are required for binding (fig. S4B). Conversely, mutation of a key residue in the nucleotide-independent interface (VpsT^{M17D}) had no effect on c-di-GMP recognition. On the basis of static multiangle light scattering, VpsT^{M17D} exists as a monomeric species in the absence of c-di-GMP, whereas intermediate molecular weights for the wild-type VpsT and the mutants VpsT^{R134A} and VpsT^{I141E} indicated fast exchange between monomers and dimers, presumably through the c-di-GMP-independent interface (fig. S5 and table S2). Addition of c-di-GMP increases the molecular weight of VpsT^{M17D} and wild-type VpsT (figs. S5 and S6), whereas the oligomeric state of VpsT^{R134A} and VpsT^{I141E} is insensitive to the nucleotide.

The role of c-di-GMP recognition and the relevance of the two dimer interfaces in DNA-binding and VpsT-regulated gene expression were assessed by using c-di-GMP binding (R¹³⁴) and dimerization (I¹⁴¹ or M¹⁷) mutants (Fig. 2). In electromobility shift assays, we used regulatory sequences upstream of *vpsL*, a gene under positive control of VpsT (Fig. 2A) (6). DNA mobility shifts were observed only for the wild-type and VpsT^{M17D} forms, where the effect was protein specific and c-di-GMP dependent. In addition, nucleotide-dependent DNA binding of VpsT was observed to multiple and relatively remote sites in the regulatory region of *vpsL*.

To evaluate the functional importance of VpsT oligomers and c-di-GMP binding in cells, we measured transcription of *vps* genes by using a chromosomal *vpsLp-lacZ* transcriptional fusion in the Δ vpsT strain harboring wild-type *vpsT*, *vpsT* point mutants (vpsT^{M17D}, vpsT^{R134A}, or

¹Department of Molecular Medicine, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA. ²Department of Microbiology and Environmental Toxicology, University of California, Santa Cruz, CA 95064, USA.

*To whom correspondence should be addressed: yildiz@metx.ucsc.edu (F.H.Y.); hs293@cornell.edu (H.S.)

vpsT^{I141E}) or the insertless expression vector (pBAD) (Fig. 2B). The presence of wild-type VpsT and VpsT^{M17D} resulted in increased *vpsL* expression, similar to that from the wild-type rugose strain carrying vector only, whereas Δ *vpsT* strains with VpsT^{R134A}, VpsT^{I141E}, or the empty vector did not exhibit such an increase. These data confirm that c-di-GMP-mediated oligomerization is critical for VpsT function. Mutations in the putative phosphorylation site designed to produce a constitutively inactive or active state, VpsT^{D60A} or VpsT^{D60E}, respectively, did not alter the efficiency of VpsT significantly. Hence, regulation of gene expression is presumably independent of phosphorylation of VpsT (see fig. S7).

Next, we determined the gene regulatory potential as a function of c-di-GMP binding and oligomerization through whole-genome expression profiling by comparing a Δ *vpsT* strain expressing either wild-type VpsT or VpsT point mutants (VpsT^{M17D}, VpsT^{R134A}, or VpsT^{I141E}) with that of cells harboring the pBAD vector alone (Fig. 2C and table S3). Genes located in the *vps*-I and *vps*-II clusters, as well as the *vps* intergenic region, were strongly induced when wild-type VpsT and VpsT^{M17D} were expressed, and significantly less so in the strains expressing VpsT^{R134A} or VpsT^{I141E}. We also observed that the expression of several genes encoding flagellar proteins was decreased in cells express-

ing wild-type VpsT and VpsT^{M17D} but not in cells expressing VpsT^{R134A} or VpsT^{I141E}, which suggested that VpsT inversely regulates motility and matrix production in a c-di-GMP-dependent manner (Fig. 2C and fig. S7). These results were corroborated in motility assays, in which a Δ *vpsT* strain or strains expressing c-di-GMP-binding mutants showed increased migration on soft agar plates compared with rugose strains that express VpsT forms that are capable of c-di-GMP-dependent dimerization (Fig. 3A).

The strain harboring VpsT^{M17D} had an expression profile similar to that of the strains harboring wild-type VpsT, however, with increased magnitude, which indicated that c-di-GMP-independent dimerization could be inhibitory or regulatory (Fig. 2, A and C). In contrast, the c-di-GMP-dependent interaction between two VpsT monomers is sufficient and necessary for DNA recognition and transcriptional regulation.

The corrugated appearance of rugose colonies can be attributed largely to increased levels of exopolysaccharides, which are induced by VpsT (6). As a consequence, *V. cholerae* mutants lacking *vpsT* produce smooth and flat colonies (Fig. 3B). To elucidate phenotypic consequences of mutations that abolish c-di-GMP binding and/or dimerization of VpsT, we compared the colony morphology of a Δ *vpsT* strain harboring wild-type VpsT with that of one of the point

mutants described above. Expression of wild-type VpsT and VpsT^{M17D} resulted in smooth-to-rugose conversion, where spot corrugation was greater in a Δ *vpsT* strain harboring VpsT^{M17D} compared with a strain with wild-type VpsT. Introduction of VpsT^{R134A}, VpsT^{I141E}, or a double-mutant VpsT^{M17D/R134A} failed to promote the smooth-to-rugose switch, but led to a distinct phenotype, characterized by increased spot diameter and weak corrugation, with a notable radial pattern (Fig. 3B and fig. S8).

Cyclic di-GMP in the rugose variant is required for increased *vps* and *vpsT* gene expression (10, 11), which suggests that VpsT is involved in a positive-feedback loop that integrates c-di-GMP to produce a robust transcriptional response. Robust matrix and biofilm formation rely on the mutual dependence of VpsT and VpsR, with VpsT introducing c-di-GMP-sensitivity to the regulatory network. In contrast, the transcriptional regulator FleQ from *Pseudomonas aeruginosa*, a distant VpsR homolog, appears to sense c-di-GMP directly, and independently of a VpsT homolog by using a distinct c-di-GMP-binding motif (18).

Taken together, we establish VpsT as a transcriptional regulator that inversely regulates biofilm formation and motility by directly integrating c-di-GMP signaling. Cyclic di-GMP-driven dimerization is mediated by an extension of the canonical receiver domains, a structural motif

Fig. 1. Crystal structure of VpsT. (A) Structure of a VpsT protomer. (B) Structure of a crystallographic trimer representing two potentially relevant, nonoverlapping dimerization interfaces. Cyclic di-GMP molecules are shown as sticks; key residues mediating ligand binding and interprotomer interactions are shown as spheres. (C) Close-up view of the nucleotide binding pocket with residues involved in coordinating the ligand shown as sticks. (Left) A ($|F_{\text{obs}}| - |F_{\text{calc}}|$) electron density map contoured at 3.6 σ is shown as calculated from a model before inclusion of c-di-GMP.

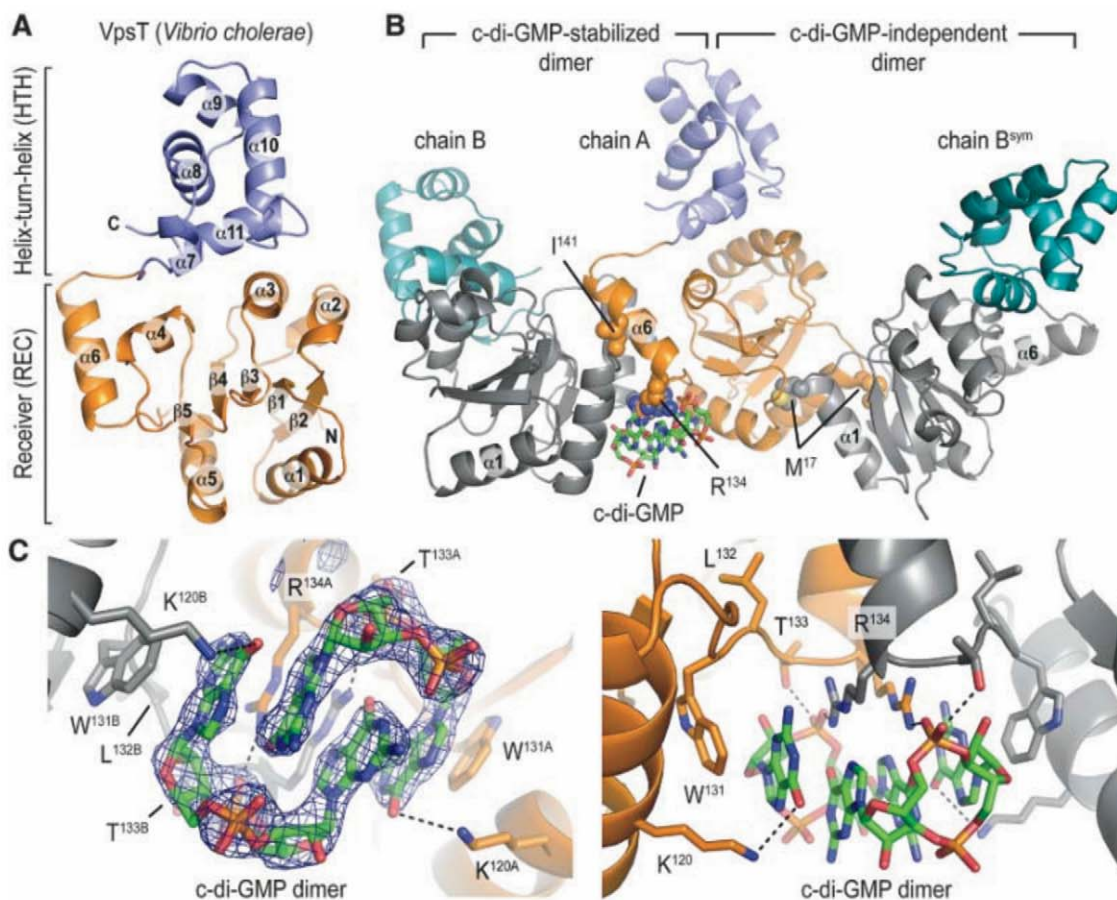


Fig. 2. Transcriptional regulation by VpsT. **(A)** Electromobility shift assays with purified proteins and biotin-labeled DNA fragments tiling the *vpsL* promoter region. Numbers indicate position relative to the open reading frame start. **(B)** *vpsL* gene expression in different genetic backgrounds harboring a single-copy chromosomal *vpsLp-lacZ* fusion. Data are means $\pm$ SD of eight replicates. **(C)** Whole-genome expression profiling. (Top) Compact heat map in a log₂-based pseudocolor scale (yellow, induced; blue, repressed) that compares a total of 108 differentially expressed genes in a $\Delta vpsT$ strain expressing wild-type (wt) or mutated VpsT versions with the vector control. (Middle) Expression profiles of genes located in and between the *vps*-I and *vps*-II clusters; (bottom) expression profiles of flagellar biosynthesis genes.

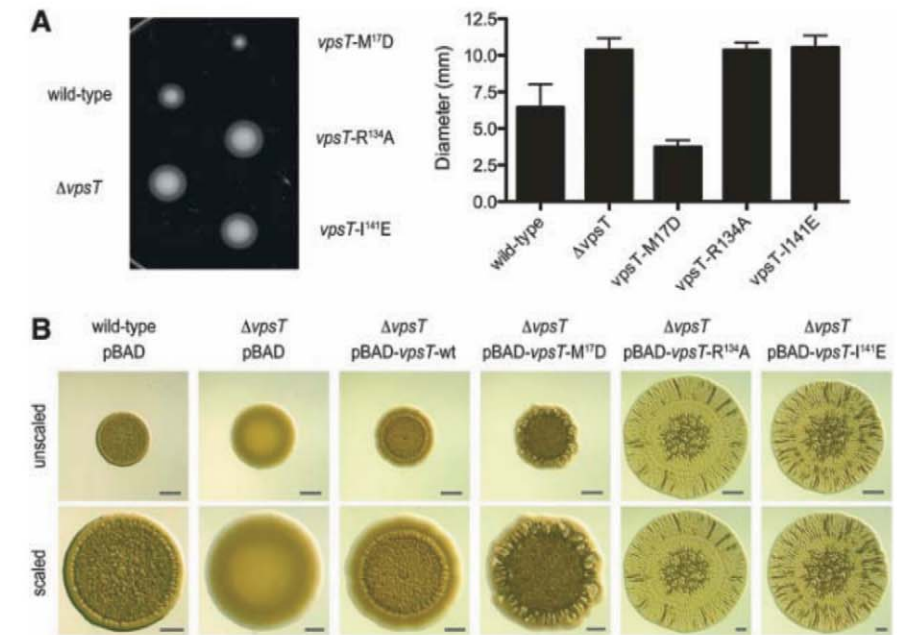
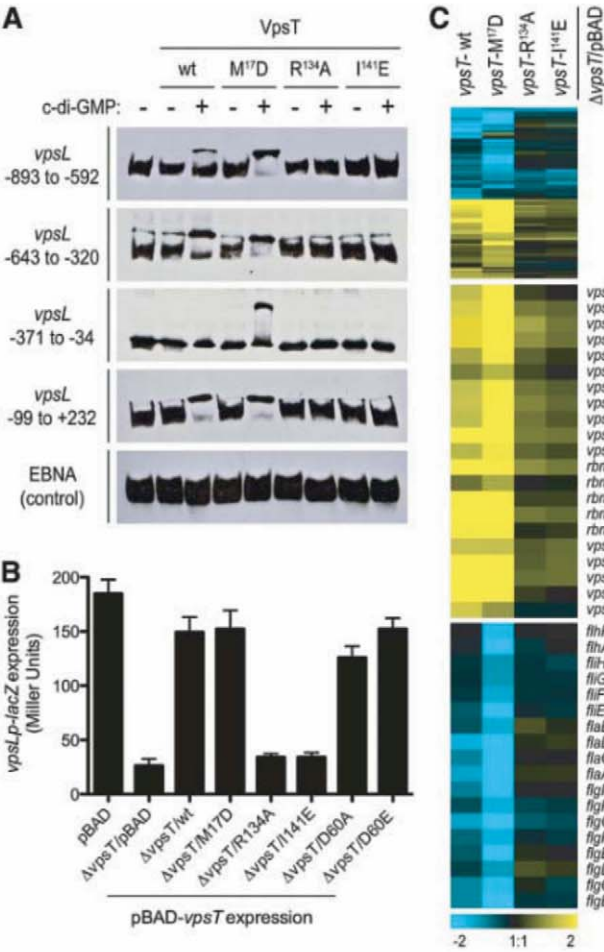


Fig. 3. Functional characterization of wild-type rugose, $\Delta vpsT$ strains, and $\Delta vpsT$ strains expressing wild-type or mutant forms of VpsT. **(A)** Motility phenotypes on semisolid Luria-Bertani broth agar plates. For strains expressing mutants of VpsT, single chromosomal insertion mutants are shown. The graph shows the mean migration zone diameter of each strain. Data are means $\pm$ SD of 11 replicates. **(B)** Spot morphologies. A wild-type rugose strain carrying the vector (pBAD) and $\Delta vpsT$ strains carrying the vector or plasmids containing wild-type or mutant *vpsT* are shown [(top) unscaled; scale bars, 2 mm; (bottom) scaled to similar diameter; scale bars, 1 mm].

that defines a widespread class of response regulators, including CsgD and other LuxR family proteins. Although some mechanisms may only pertain to close homologs of VpsT, such as c-di-GMP-dependent dimerization, the general mode of action involving dimerization accompanied by changes in the relative orientation of the DNA binding domains is likely to be relevant for the large family of homologous transcription factors.

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14. Materials and methods are available as supporting material on Science Online.
15. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. The single point mutation R134A is a substitution of Ala for Arg¹³⁴.
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19. We are grateful to S. Hubbard and W. Horne for providing access to light scattering and calorimetry, respectively, and to the staff at the National Synchrotron Light Source (NSLS; Brookhaven National Laboratories) for assistance with synchrotron data collection. The NSLS is supported by the Offices of Biological and Environmental Research and of Basic Energy Sciences of the U.S. Department of Energy, and by the National Center for Research Resources of the NIH. This work was supported by the NIH (1R01GM081373 to H.S. and R01AI055987 to F.H.Y.), and by a PEW Scholar award (H.S.). Atomic coordinates and structure factors have been deposited in the RCSB Protein Data Bank under ID code 3KLN and 3KLO. The microarray data have been deposited in NCBI's Gene Expression Omnibus and are accessible through GEO series accession number GSE19479.

Supporting Online Material
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Materials and Methods
SOM Text
Figs. S1 to S13
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Darwinian Evolution of Prions in Cell Culture

Jiali Li,* Shawn Browning,* Sukhvir P. Mahal, Anja M. Oelschlegel, Charles Weissmann†

Prions are infectious proteins consisting mainly of PrP^{Sc}, a β sheet-rich conformer of the normal host protein PrP^C, and occur in different strains. Strain identity is thought to be encoded by PrP^{Sc} conformation. We found that biologically cloned prion populations gradually became heterogeneous by accumulating “mutants,” and selective pressures resulted in the emergence of different mutants as major constituents of the evolving population. Thus, when transferred from brain to cultured cells, “cell-adapted” prions outcompeted their “brain-adapted” counterparts, and the opposite occurred when prions were returned from cells to brain. Similarly, the inhibitor swainsonine selected for a resistant substrain, whereas, in its absence, the susceptible substrain outgrew its resistant counterpart. Prions, albeit devoid of a nucleic acid genome, are thus subject to mutation and selective amplification.

Prions are the infectious agents responsible for a variety of neurodegenerative disorders, including scrapie in sheep, bovine spongiform encephalopathy (BSE) in cattle, and new variant Creutzfeldt-Jacob disease (CJD) and kuru in humans. The principal, if not only, component of the prion is PrP^{Sc}, a β sheet-rich conformer of prion protein, (PrP). PrP^{Sc} propagates by eliciting conversion of PrP^C, the physiological form of PrP, into a likeness of itself. The seeding hypothesis posits that PrP^C is in equilibrium with PrP^{Sc} or a PrP^{Sc} precursor, with the equilibrium largely in favor of PrP^C, and that PrP^{Sc} is only stabilized when it forms an aggregate, or seed, containing a critical number of monomers. Once a seed is present, monomer addition ensues rapidly (1).

Prions occur in the form of distinct strains, originally characterized by the incubation time and the neuropathology they elicit in a particular host (2). Many different strains can be propagated indefinitely in hosts homozygous for the PrP gene (*Prnp*); the protein-only hypothesis assumes that each strain is associated with a different conformer of PrP^{Sc} (3–5), which implies that there are as many stable conformations of PrP as there are stable prion strains that can be propagated in a particular mouse strain, perhaps 15 or more (6). The concept of “conformation templating” at the protein level was first supported by cell-free conversion experiments (7) and extended by the development of protein misfolding cyclic amplification (PMCA), which mimics PrP^{Sc} autocatalytic replication *in vitro* (8).

A prion strain, transferred from one species to another and subsequently returned to the original host, may in some instances have changed or “mutated” (9, 10). Novel strains may arise not only by mutation of naturally

occurring strains, but also *de novo*, in transgenic mice (11) or in cell-free systems, mediated by PMCA (12).

Strains are classically differentiated by mouse bioassays that require months or years to complete. The cell panel assay (CPA) (13) allows assessment of the characteristic cell tropism of strains by the standard scrapie cell assay (SSCA) (14) on a panel of four cell lines, the neuroblastoma-derived PK1 and R33, the neuronal CAD, and the fibroblastic LD9 lines. The CPA readily distinguishes between RML, 22L, ME7, and 301C prions within about 2 weeks.

We have found that 2 μ g/ml (11.55 μ M) swainsonine (swa), an inhibitor of Golgi α -mannosidase II that impairs formation of complex N-linked glycans, inhibits by 99% or more chronic infection of PK1 cells by RML and 79A but not by 22L prions. The median effective dose for inhibition of infection is 3 ng/ml. The misglycosylation of host proteins caused by swa has no effect on cell growth but reduces the accumulation rate of “swa-sensitive,” but not of “swa-resistant,” prions below the replication rate of the host cells, which causes the prions to be diluted out with progressive cell doublings.

It has been reported that strain specificity is retained when prions are transferred from brain to cultured cells and back to brain (15, 16); however, the properties of the prions while in cell culture could not be determined by classical procedures. We therefore examined prion characteristics using the CPA. We generated a chronically infected cell population, PK1[22L]_{wp}, by exposing PK1 cells to 22L-infected mouse brain homogenate (brain[22L]) and propagating them for about 34 doublings. The CPA showed that cell-derived and brain-derived prions differed: The cell-derived prions were unable to infect R33 cells (“R33 incompetent”) or to infect PK1 cells in the presence of swa (“swa sensitive”), in contrast to brain[22L]-derived prions, which were able to do both. (Fig. 1, A and C). We considered that cell- or brain-derived components might influence the infectious properties of the prions on

the cell panel. If this were so, the full change in properties would be observed immediately after the first round of prion replication in the cells. We therefore infected PK1 cells with 22L-infected brain homogenate and collected conditioned medium 9 days after infection (P0) and after successive 1:10 splits. The CPA (Fig. 1B) showed that, at P0, the secreted prions resembled brain prions, in that they were R33 competent and swa resistant, and that with successive splits they became less infectious to R33 and more susceptible to inhibition by swa. By the 12th 1:10 split, i.e., after about 40 doublings, the properties of the population were indistinguishable from those of the PK1[22L]_{wp}. Thus, when transferred from brain to PK1 cells, the prion population underwent a gradual, not a sudden, change in properties. This suggested that the brain-derived population might be heterogeneous and that the R33-competent, swa-resistant prions that predominated in brain were replaced by R33-incompetent, swa-sensitive prions with a growth advantage in PK1 cells.

Having established that the prions in PK1[22L]_{wp} cells were swa sensitive, we attempted to cure the cells of infection by propagating them in the presence of swa for ten 1:20 splits (Fig. 2, A and B). In the absence of swa, the percentage of PrP^{Sc}-positive cells remained essentially unchanged, between 30 and 40%. In the presence of swa, the percentage initially dropped, from 35% to about 7%, but then, unexpectedly, increased to reach a value of about 25% by the 8th to 10th split, which suggested the development of resistance. To investigate this in more detail, we analyzed the swa susceptibility of prions secreted by PK1[22L]_{wp} cells. Prions from cells propagated in the absence of swa were swa sensitive at all splits tested (Fig. 2C). In contrast, prions from cells propagated in the presence of swa for two or more splits were completely swa resistant. In addition, PK1[22L]_{wp} cells grown for five passages in the presence of swa and then for five splits in its absence secreted prions that were again fully swa sensitive. Thus, in the presence of the drug, preexisting or newly generated swa-resistant prion variants selectively grew to dominate the population. Moreover, after withdrawal of the inhibitor, residual or newly generated swa-sensitive prions replaced their swa-resistant counterparts almost completely after about 22 doublings, presumably because, in the absence of swa, the drug-sensitive prions replicated more rapidly. We confirmed all these results by assaying lysates of the same cell cultures described above (17). Moreover, the entire experiment was repeated by a different operator with the same results.

Swa prevents normal complex glycosylation of N-linked glycans (18), and the resulting high-mannose glycans are cleavable by endoglycosidase H (Endo H), in contrast to native complex glycans, which are completely resistant. Treatment of proteinase K (PK)-digested samples from control PK1[22L]_{wp} cells (Fig. 2D) with Endo H

Department of Infectology, Scripps Florida, 130 Scripps Way, Jupiter, FL 33458, USA.

*These authors contributed equally to this work.

†To whom correspondence should be addressed. E-mail: charlesw@scripps.edu

did not result in PrP bands with increased mobility. However, after the first 1:20 split (about 4.3 doublings) in swa-containing medium (Fig. 2D), there was a significant mobility shift of all bands due to the loss of complex glycosylation, and Endo H treatment caused a dramatic increase in mobility. Yet, as described above (Fig. 2C and table S1), the prions from this sample were still swa sensitive, which showed that the lack of complex glycosylation was not the cause of swa resistance. When swa treatment was discontinued, the mobility pattern reverted to that of untreated controls after two 1:20 splits [SC7 in (Fig. 2D)], and the glycans were completely resistant to Endo H. However, the prions continued to be swa resistant until after the fifth

split (SC10). Thus, lack of complex glycosylation is not responsible for swa resistance, and normally glycosylated PrP^{Sc} can be associated with both swa-sensitive and swa-resistant prion variants.

Some prion strains differ in the site at which the cognate PrP^{Sc} is cleaved by PK (3, 4, 19) or thermolysin (20) or in the rate at which it is degraded (21). We treated lysates of swa-sensitive and swa-resistant PK1[22L]_{wp} cells with PK or thermolysin (fig. S3) and detected no significant differences in the electrophoretic mobility of the cleavage products. There was no significant difference in the susceptibility of PrP^{Sc} from brain[22L], swa-sensitive, or swa-resistant PK1[22L]_{wp} cells to PK (fig. S4). PrP^{Sc} from different strains may

exhibit different stabilities, as determined by susceptibility to PK digestion after exposure to increasing concentrations of guanidinium chloride (Gnd.HCl) (11, 22). We subjected lysates of swa-sensitive and swa-resistant PK1[22L]_{wp} cells, as well as homogenates of brain[22L], to the conformational stability assay, but found no significant differences in the Gnd.HCl_{1/2} values, which were 1.1 to 1.3 M in all cases (fig. S5). These negative findings suggest that structural differences between the postulated substrains may be discrete.

Cell lysates were injected intracerebrally into C57BL/6 mice, and brains were recovered when the mice became terminally ill at 147 days after inoculation (table S2). The CPA of authentic brain[22L] and the brain-passaged swa-sensitive and swa-resistant PK1[22L]_{wp} prions gave indistinguishable patterns (Fig. 1C and fig. S6), showing that the PK1-derived 22L prions regained their original cell tropism after propagation in brain. Brain sections revealed the vacuolization of the granular layer of the cerebellum and the loss of Purkinje cells typical for 22L for both cell-derived (swa-sensitive and swa-resistant) and brain-derived samples (fig. S7 and table S3).

The finding that exposure of 22L-infected PK1 cells to swa leads to the emergence of swa-resistant prions means that such variants either exist in the population at a low level before exposure to the drug. To address this question, we exposed PK1 cells to PK1[22L]_{wp} prions in either the presence or absence of swa for 2 days and distributed the cells into 96-well plates at 8 cells per well for the cells infected in the absence of swa and 1000 cells per well for cells infected in the presence of swa (fig. S2). Uninfected cells were added to bring the total number of cells in each well to 1000. The cells were grown to confluence and split 1:10 five times, in the continued absence or presence of swa. Under these conditions, any well containing one or more infected cells yields a positive signal in the PK-digested enzyme-linked immunosorbent assay (PK-ELISA) (17) because of the continuous spread of infection (14). The average number of infected cells delivered to each well was calculated by the Poisson equation. Of the wells from the swa-exposed population, 0.029% were PrP^{Sc}-positive, and from the non-swa-exposed population, 5.8% were PrP^{Sc}-positive (fig. S2). Because swa inhibits replication of PK1[22L]_{wp} prions by more than 99%, these results indicate that 0.5% of the prions secreted by PK1[22L]_{wp} cells were swa resistant before exposure to the drug.

The 22L isolate obtained from the TSE Resource Center (Compton, UK) had been biologically cloned twice in succession (23), yet here we found it to be heterogeneous with regard to its swa sensitivity after transfer to cultured cells, which suggested that variants had arisen during the two rounds of propagation in mice and the transfer to

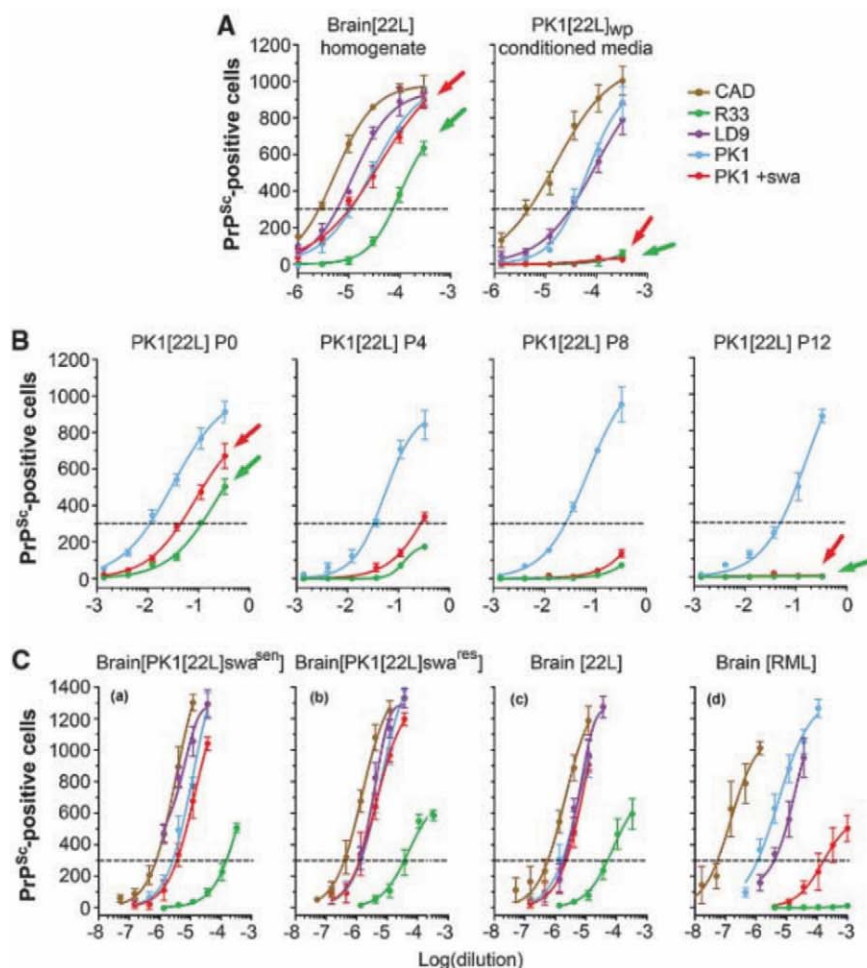


Fig. 1. Prion characterization by the CPA. Cells were exposed to the serially diluted samples indicated. The proportion of PrP^{Sc}-positive cells was plotted against log₁₀ dilution. (A) Brain[22L]-derived prions are swa resistant and R33 competent, whereas those from 22L-infected PK1 cells (about 27 doublings after infection) are swa sensitive and R33 incompetent. (B) Transfer of prions from brain to PK1. PK1 cells were exposed to 10⁻⁴ brain[22L] for 1 day and propagated 9 days (P0); further propagation for twelve 1:10 splits yielded P1 to P12. Conditioned media were analyzed on PK1 cells with (red) or without (blue) 2 μ g/ml swa and on R33 cells (green). Infection in the presence of 10 μ g/ml pentosan polysulfate, which abolishes prion replication, yielded no infectivity, documenting the absence of inoculum. (C) Brain homogenates from terminally ill C57BL/6 mice inoculated with lysates of PK1[22L]_{wp} cells propagated in the absence (a) or presence (b) of swa or with (c) 1% 22L-infected brain homogenate gave the same CPA responses. (d) The very different CPA response of brain RML is shown for comparison.

cells. To ascertain whether cloned prion populations could become heterogeneous, we recloned 22L prions by end-point dilution in cell culture (16) and analyzed the populations after various extents of propagation (Fig. 3). Eight clonal populations were assayed after about 31 doublings following infection and found to secrete swa-sensitive prions (fig. S8). Aliquots of each population were propagated for 22 doublings in the presence or absence of swa; two of the eight clones, 8C4 and 3C6, secreted swa-resistant

prions after being grown in the presence, but not in the absence, of the drug (Fig. 3A). The other six cell clones secreted swa-sensitive prions (Fig. 3A) and lost infectivity after being propagated for five 1:20 splits in the presence of swa (Fig. 3A). Three of the six clones (8A8, 8B4, and 8H6) were propagated for an additional 22 doublings in the absence of the drug [(b) to (c1) in Fig. 3A], altogether about 53 doublings, and were then exposed for 22 doublings to swa, whereupon one more clone (8A8) produced swa-

resistant prions (Fig. 3A). Thus, swa-resistant variants arose during propagation of cloned, swa-sensitive prion populations for as few as 31 doublings in the absence of the drug. From these data, we calculated (17) a very approximate “mutation rate” of 10^{-6} per doubling. This number is an underestimate, mainly because we considered neither the selective disadvantage of swa-resistant prions in the absence of swa nor the “reversion rate.”

Perhaps swa resistance and R33 competence are only two of many variations that can arise in a 22L population; if so, the overall mutation rate could be even greater and the prion population more diverse, comprising a multiplicity of “substrains” or “types” (24). This would be reminiscent of the “quasispecies” concept (25, 26). Although heterogeneity in the case of RNA viruses is due to point mutations resulting from error-prone replication, heterogeneity in the case of the prions is likely to be due to differences in the structure (other than the amino acid sequence) of the PrP^{Sc} molecules. These differences could reflect variations in the conformation of the PrP^{Sc} resulting during conversion; the conformational changes may be subtle, but sufficient to facilitate propagation in a particular environment. Alternatively, or in addition, variation could be due to cell-derived determinants (for example, association of PrP with a small cellular RNA) or to the nature of its glycosylation.

Transfer of a prion strain from one animal species to another usually entails a low attack rate and long incubation times, which in subsequent passages are dramatically reduced (27). If the only barrier to prion transfer between species were the initial round of heterologous conversion, then once it occurred, propagation would be rapid. However, this is almost never so, and two or three sequential transmissions are required to obtain stable, shortened incubation times, which suggests that additional, likely conformational, changes are required to optimize prions for propagation in the new host (9, 10, 28). It has also been argued that even within a single host, different “strain types” may develop within different tissues (29–31), and the “cloud” model to explain these observations (24) is well supported by our findings.

In what ways do the concepts of strains and substrains differ? The energy landscape diagram of fig. S10 depicts the view that substrains are distinct collectives of prions that can interconvert reproducibly and relatively readily, that is, within the generation time of the host or a few dozen rounds of replication, because they are separated by relatively low activation energy barriers; strains, each comprising a set of readily interconvertible substrains, are separated by higher energy barriers, causing transitions to be rare events.

In summary, prions show the hallmarks of Darwinian evolution: They are subject to mutation, as evidenced by heritable changes of their

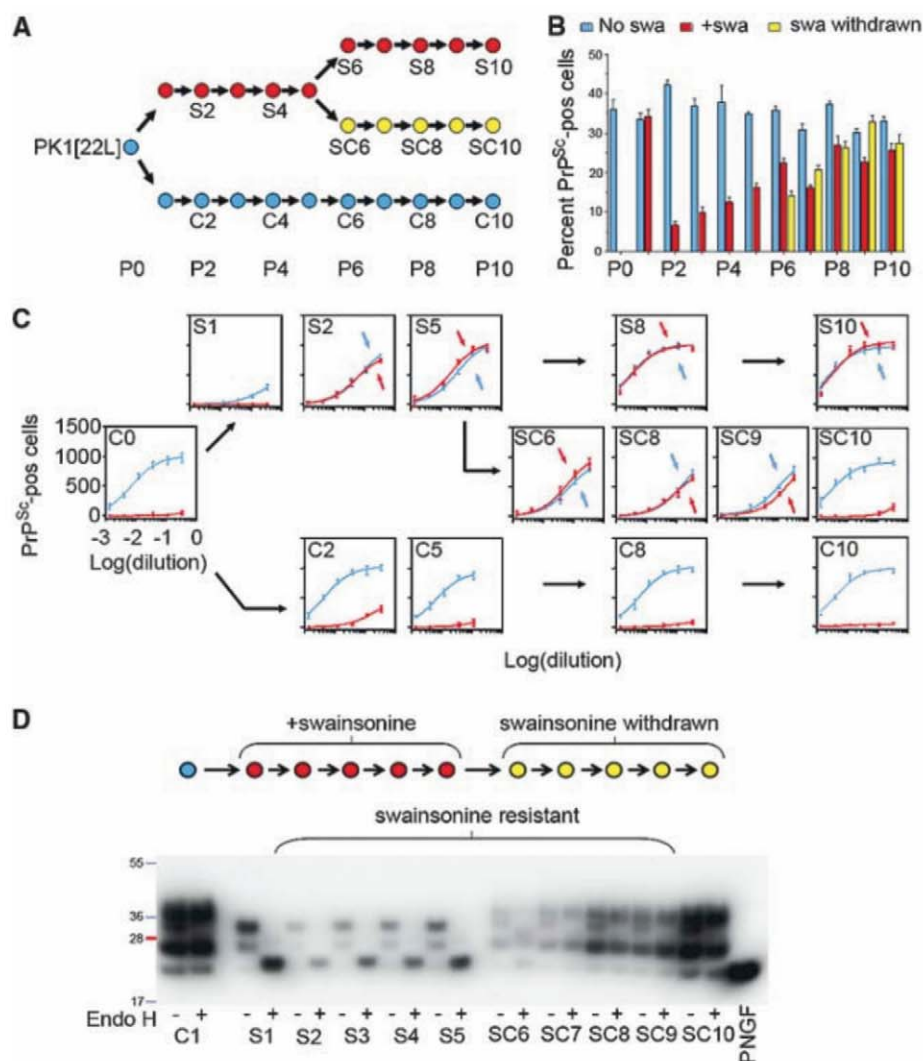


Fig. 2. Propagation of 22L-infected PK1 cells in swa results in swa-resistant prions. (A) Scheme. PK1[22L]_{wp} cells were either grown in swa (2 μ g/ml) for up to 10 splits (S1 to S10), without swa for up to 10 splits “C0–C10”, or with swa for 5 splits followed by without swa for 5 splits (SC6 to SC10). (B) The percentage of PrP^{Sc}-positive cells propagated with swa (red) or without swa (blue, yellow), as assessed by PK-ELISA. (C) Swa susceptibility of secreted prions propagated in the presence or absence of swa. Conditioned medium, concentrated 100 $\times$, was assayed on PK1 cells in the presence (red) or absence (blue) of swa. (D) Swa-resistant prions are associated with both PrP^{Sc} carrying high-mannose glycans (S2 to S5) and normally glycosylated PrP^{Sc} (SC7 to SC8). PK1[22L]_{wp} cell lysates digested with PK and Endo H were subjected to immunoblotting with antibody against PrP. After the first split in swa, PrP bands shift to higher mobility, which reflects the inhibition of complex glycosylation, and Endo H digestion results in a large mobility increase due to removal of high-mannose glycans. After two splits without swa (SC7), normal glycosylation is restored but swa resistance is retained.

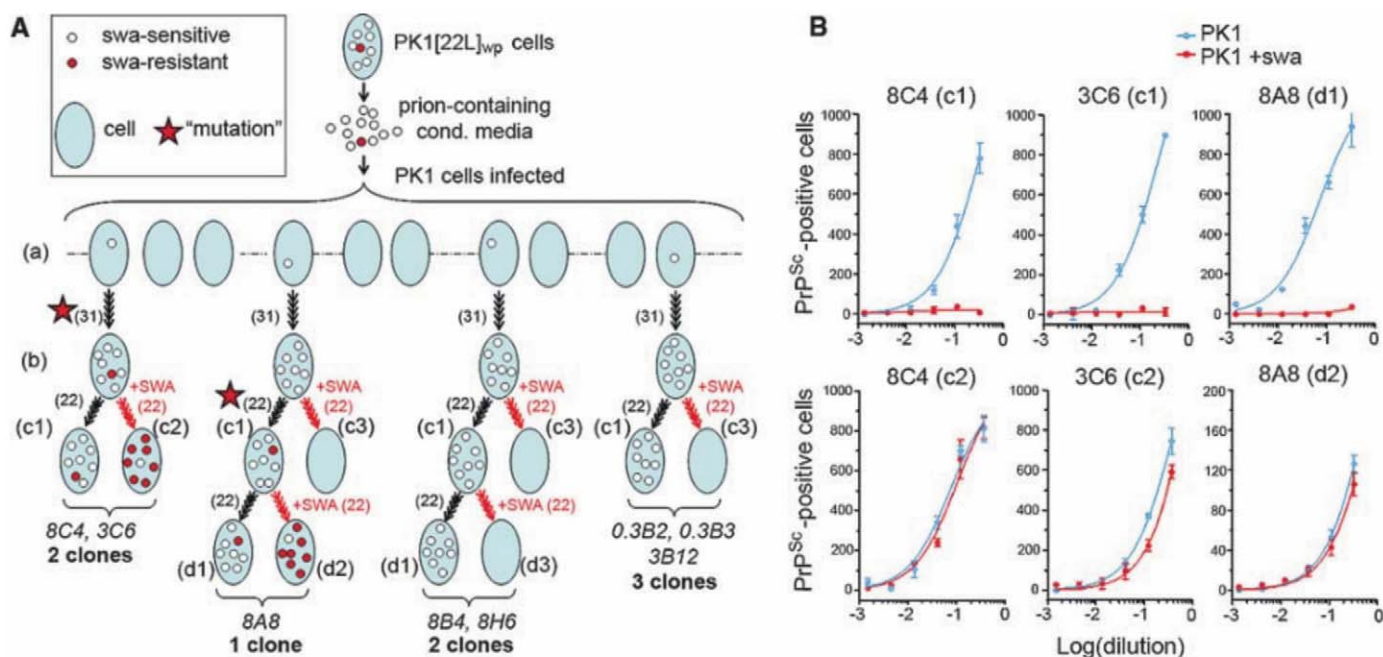


Fig. 3. Development of heterogeneity in cloned prion populations. Prions were cloned by end-point dilution in cell culture, and eight clones were tested for their ability to yield swa-resistant populations after propagation in swa. Values in parentheses indicate number of doublings. **(A)** PK1 cells were exposed to PK1[22L]_{wp} conditioned medium for 2 days, which led to infection of about 4% of the cells. Cells were distributed at 0.3, 1, 3, or 8 cells per well (a), along with about 1000 uninfected cells, grown to confluence (7 doublings) and split 1:10 five times (17 doublings) in 96-well plates. Of 602 wells, 23 scored positive by PK-ELISA. Eight clones were expanded for seven doublings, and all secreted swa-sensitive prions

[(b) altogether 31 doublings]. After propagation in swa for five 1:20 splits, clones 8C4 and 3C6 yielded swa-resistant prions (c2), whereas the others were cured (c3). Propagation without swa yielded swa-sensitive prions (c1). Three of six clones that failed to yield swa-resistant populations after exposure to swa (8A8, 8B4, and 8H6) were further passaged for 22 doublings without swa (c1), followed by 22 doublings with swa, whereupon one (8A8) yielded swa-resistant prions (d2), and two (8B4 and 8H6) were cured (d3). Details in (17). **(B)** Swa resistance was determined by assaying 100× concentrated conditioned medium on PK1 cells with or without swa.

phenotypic properties, and to selective amplification, as documented by the emergence of distinct populations in different environments. A practical consequence of our findings is the realization that therapeutic approaches aimed at stabilizing PrP or reducing PrP expression are less likely to be thwarted by emergence of drug resistance than those based on targeting PrP^{Sc}.

Note added in proof: Exposure of mice or differentiated neuroblastoma cells infected with RML prions to quinacrine leads to drug-resistant prions (32).

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Supporting Online Material

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Materials and Methods
SOM Text
Figs. S1 to S10
Tables S1 to S4
References

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Repulsion of Superinfecting Virions: A Mechanism for Rapid Virus Spread

Virginie Doceul,* Michael Hollinshead,* Lonneke van der Linden,† Geoffrey L. Smith‡

Viruses are thought to spread across susceptible cells through an iterative process of infection, replication, and release, so that the rate of spread is limited by replication kinetics. Here, we show that vaccinia virus spreads across one cell every 75 minutes, fourfold faster than its replication cycle would permit. To explain this phenomenon, we found that newly infected cells express two surface proteins that mark cells as infected and, via exploitation of cellular machinery, induce the repulsion of superinfecting virions away toward uninfected cells. Mechanistically, early expression of proteins A33 and A36 was critical for virion repulsion and rapid spread, and cells expressing these proteins repelled exogenous virions rapidly. Additional spreading mechanisms may exist for other viruses that also spread faster than predicted by replication kinetics.

Mechanisms enhancing the cell-to-cell spread of intracellular pathogens are important for virulence and are targets for development of antimicrobial therapeutics. Vaccinia virus (VACV) is a poxvirus and is the live vaccine used to eradicate smallpox (1). VACV replication is unusual in that it produces both single- and double-enveloped virions (2, 3). The single-enveloped virions, called intracellular mature virus (IMV), remain intracellular until cell lysis and spread slowly from cell to cell. In contrast, the double-enveloped virions, called cell-associated enveloped virus (CEV) and extracellular enveloped virus (EEV), are released rapidly and mediate efficient cell-to-cell spread and long-range dissemination (3, 4). VACV spreading mechanisms include virus-induced cell motility (5) and the formation of actin projections (6–8) that propel VACV particles toward other cells late during infection (9). However, we wondered whether either mechanism could explain how VACV Western Reserve (WR) spreads rapidly to form a plaque of diameter 2.90 ± 0.07 mm (SEM, nine experiments, $n = 11$ to 12 plaques) in 3 days (Fig. 1A). The distance between nuclei of adjacent BSC-1 cells was 37.26 ± 1.02 μ m (SEM, $n = 25$ single cells and the 5 to 8 cells in contact with it), so that VACV was spreading across each cell in <2 hours. To study this further, live video microscopy was used to measure the spread of VACV-induced cytopathic effect after infection with VACV WR (Fig. 1, B and C) or VACV expressing enhanced green fluorescent protein (EGFP) fused to core protein A5 that is expressed late during infection (vEGFPA5L) (Fig. 1, D to F, and movies S1 to S5) (10, 11). A linear increase in plaque size with time was observed (Fig. 1G), and the mean rate of spread was 32.36 ± 0.74 μ m/hour (SEM, $n = 9$ plaques)

for VACV WR. Knowing the distance between nuclei of adjacent cells, this indicated that VACV crossed one cell every 1.2 hours. This rate of spread is inconsistent with VACV replication kinetics, in which new virions are formed only 5 to 6 hours after infection (12), or virus-induced

cell motility, in which cells start to move 5 to 6 hours after infection (5). These observations demonstrated that another mechanism to accelerate spread must exist. Mutants defective in actin tail formation (Δ A36R, Δ A33R, Δ A34R, Δ B5R, Δ F13L, and Δ F12L) (3) spread much more slowly (Fig. 1, G and H), infecting only one cell every 5 to 6 hours, which is consistent with replication kinetics. This reaffirmed the importance of actin tails for VACV spread but did not explain the rapid dissemination because actin tails are produced only late during infection after new virions are formed.

Inspection of plaques formed by vEGFPA5L revealed EGFP-positive virions (green dots) several cells away from EGFP-positive cells, where virions are formed, showing that VACV particles spread rapidly to distal cells (Fig. 1F). To investigate this phenomenon, actin was stained with phalloidin, and confocal optical sections revealed virus-tipped actin tails on cells producing new virions but also on distal cells lacking virus factories and so not producing virions (no

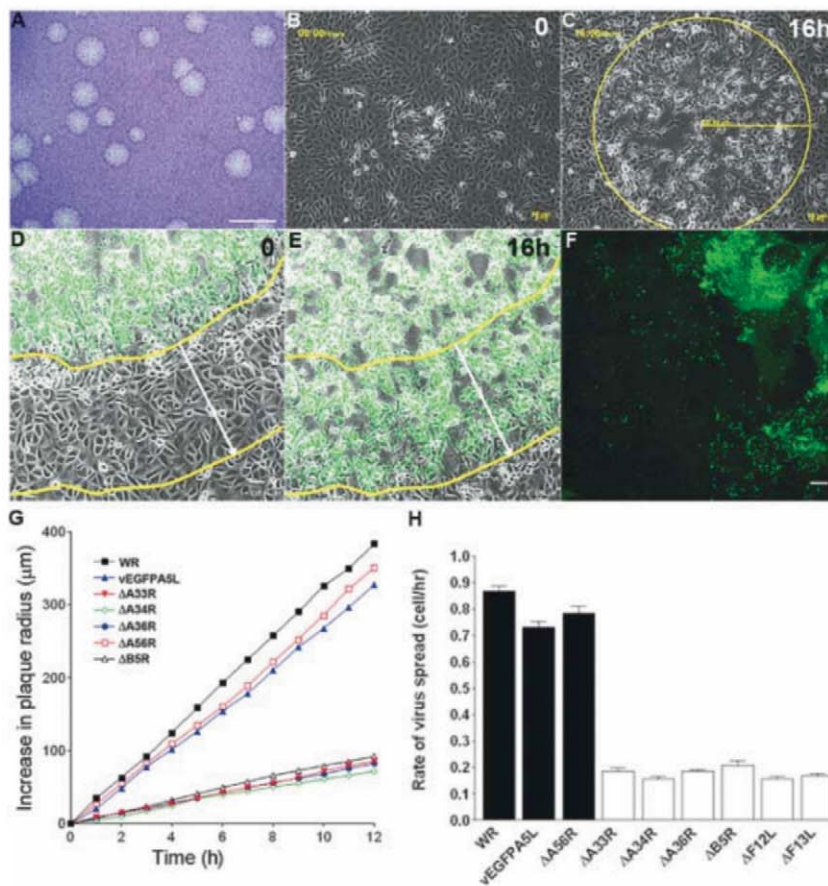


Fig. 1. VACV spreads more rapidly than predicted. (A) VACV plaques 3 days after infection in BSC-1 cells. Scale bar, 5 mm. (B and C) Live cell imaging recording plaque formation at 0 (B) and 16 (C) hours later. (D and E) Live cell imaging of vEGFPA5L-infected cells confirmed the correlation between cytopathic effect (cpe) and virus infection. Yellow lines indicate the boundary between infected and uninfected cells and white arrows indicate the distance this has moved over 16 hours. (F) Confocal image showing the spread of EGFP-tagged virus particles (single green dots) far from the center of infection. (G) Increase in plaque radius formed by VACV WR and mutants with time; $n = 6$ to 11 plaques. (H) Diagram showing the rate of spread (BSC-1 cell per hour) with indicated viruses. White bars indicate viruses with a defect in actin tail formation. Error bars are SEM, with $n = 6$ to 11 plaques. Scale bar, (A) 5 mm, (B) to (E) 50 μ m, and (F), 10 μ m.

Department of Virology, Faculty of Medicine, Imperial College London, St Mary's Campus, Norfolk Place, London W2 1PG, UK.

*These authors contributed equally to this work.

†Present address: Radboud University Nijmegen, Nijmegen Centre for Molecular Life Sciences, 6500 HB Nijmegen, The Netherlands.

‡To whom correspondence should be addressed. E-mail: geoffrey.lsmith@imperial.ac.uk

EGFP expression) (Fig. 2A). This result was reproduced in different cell lines (BSC-1, RK13, ECV, and CEF) and using different VACV strains (WR and Lister) (figs. S1 and S2). To be certain that actin tails on cells that lack virus factories were not derived from distant virus-producing cells, a lawn of cells was formed in which some cells expressed cherry fluorescent protein fused to actin (cherry-actin) and thus produced red actin tails after virus infection (Fig. 2B and movies S6 and S7). After infection of such monolayers at low multiplicity, cells containing green factories adjacent to a red cell (cherry-actin positive) were

studied. This revealed green virions on tips of red actin tails originating from a red cell that lacked any green factory and therefore new virions. Careful examination of *z* stacks of these cells confirmed no virus factory was present. Therefore, the virions and actin tail originated from different cells. Further examination by means of time-lapse microscopy revealed virus-tipped red actin tails on a cell 5, 20, and 50 min before green factory formation (55 min) (Fig. 2B), confirming that actin tails appeared before virion production. Furthermore, virions on a red actin tail were observed recontacting the same red cell and induc-

ing another actin tail (movie S7). Thus, virions can be repelled repeatedly, thereby accelerating spread until an uninfected cell is found.

To understand this mechanism, we searched for the required VACV proteins. Proteins K2 and A56 inhibit entry of IMV by binding the membrane fusion complex on the IMV surface (13–15), but this is masked on EEV or CEV, and mutants lacking these genes form normal-sized plaques (16, 17). Better candidates would be early, cell-surface proteins that are needed for actin tail formation. Although proteins A33, A34, A36, B5, F12, and F13 are needed for efficient actin tail

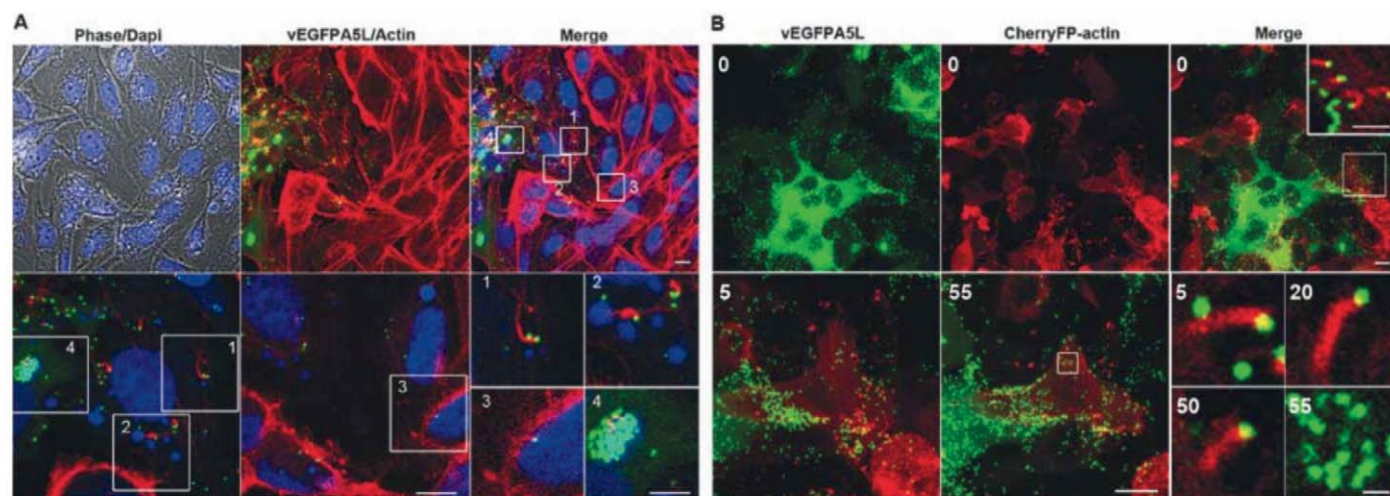


Fig. 2. Cells form actin tails before production of new virions. **(A)** Confocal images showing the edge of vEGFPA5L plaque (green) on BSC-1 cells stained for actin (red) or DNA (blue). Bottom panel shows zoomed areas (white squares 1 to 4). Actin tails are on cells with nascent factories (cytoplasmic blue) but that are not producing any virus particles (green) (squares 1 and 2), and on a cell with no virus factory (square 3), whereas square 4 shows a productive virus factory (green). Scale bars, top row, 10

μm ; bottom row, 10 μm ; and insets 1 to 4, 5 μm . **(B)** Actin tails (red) present at the surface of a cell expressing cherryFP-actin but with no green virus factory [time (*t*) = 0, white square and zoomed inset]. Bottom panels show zoomed images of this cell with actin tails detected 5, 20, and 50 min later, before the appearance of virus factories at 55 min as indicated by the white square. Scale bars, top row, 10 μm ; top right inset, 5 μm ; bottom row, 10 μm ; and bottom right inset, 1 μm .

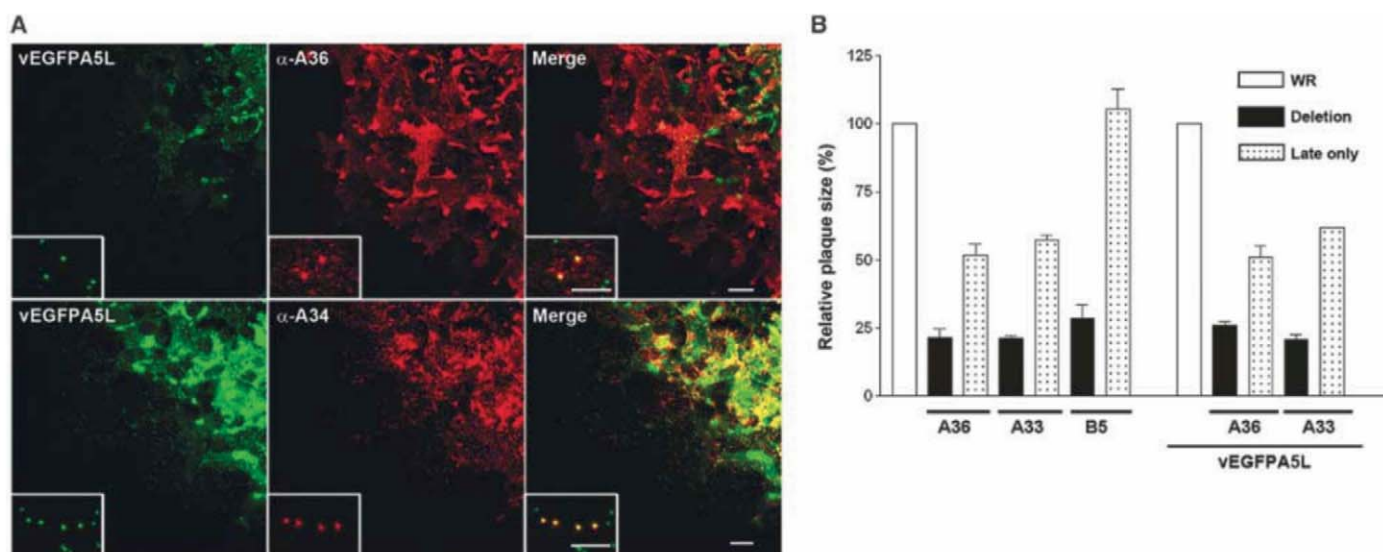


Fig. 3. Early expression of A33 and A36 is important for VACV spread. **(A)** Images of edge of plaque showing A36, but not A34, is expressed early during infection. A36 was detected in cells where no late protein A5 (green) was present, whereas A34 was expressed late during infection in cells that also express A5. (Insets) Zoomed images of virions (single green dots)

relative to A36 and A34 distribution. **(B)** Graph showing the size of plaques formed by recombinant viruses in which A33R, A36R, or B5R are under a late promoter only (4b) or deleted (Δ) as compared with parental viruses WR or vEGFPA5L. Error bars are SEM mean values from three experiments with *n* = 11 to 12 plaques. Scale bars, 20 μm ; insets, 5 μm .

formation, only surface proteins B5 (18), A33 (19), and A36 (20) are expressed early (and late). Immunostaining of plaques formed by vEGFPA5L demonstrated that A33 and A36 are expressed early during infection at the periphery of plaques before expression of late proteins (A34, EGFP-A5, and B5) (Fig. 3A and fig. S3). A34 and B5 were nevertheless detectable on virions spreading toward noninfected cells, as expected. Thus, A33 and A36 seemed candidates for early induction of actin tails.

The importance of early expression of A33 and A36 was investigated by generating recom-

binant viruses in which *A33R*, *A36R*, or *B5R* genes were driven only by a late promoter (21). Infection of cells by these viruses (v4b-A33, v4b-A36, and v4b-B5) confirmed expression of these proteins only late during infection (fig. S4) and showed that plaques formed by v4b-A33 and v4b-A36 were much smaller than wild type and closer to those formed by deletion mutants lacking either gene (Fig. 3B). In contrast, v4b-B5 formed plaques similar to wild type. Thus, early expression of A33 and A36, but not B5, is critical for efficient VACV spread. Similar results were obtained with viruses in which 4b-*A33R* and 4b-

A36R were inserted into vEGFP-A5L, allowing direct visualization of spreading virions via EGFP. Virions released from cells infected by these viruses (vEGFPA5L/4b-A33 and vEGFPA5L/4b-A36) spread poorly as compared with vEGFPA5L and induced actin tails only on cells with a virus factory (fig. S5).

Next, we investigated whether A33 and A36 are sufficient to induce actin tails upon contact with an EEV particle. Both proteins are expressed on the plasma membrane of VACV-infected cells (22–24) and interact with each other (25–27). Lentivirus vectors expressing A33 or A36 fused to a C-terminal V5 tag were used to generate HeLa cells expressing cell surface A33-v5, A36-v5, or A36-v5 and A33-v5 (Fig. 4A and fig. S6). These cells were incubated with EEV particles and then stained with phalloidin and a monoclonal antibody to B5. Actin tails were detected on cells expressing A33-v5 and A36-v5 within 15 to 30 min (Fig. 4, B and C). Similar results were obtained with haemagglutinin (HA)-tagged A33. No actin tails were detected if only one protein was expressed or if the cells were incubated with IMV or with GFP-tagged herpes simplex virus 1 (HSV-1). Thus, A33 and A36 are both necessary and sufficient to induce actin tails after binding EEV particles. Currently, we are investigating the effect of ectopic expression of A33 and A36 on VACV spread by measuring plaque size using additional cell lines that form clearer plaques.

Here, we demonstrate that VACV has evolved a mechanism (Fig. 4D) by which infected cells repel superinfecting CEV/EEV particles on actin tails toward neighboring cells. Two outcomes are then possible: (i) If the neighboring cell is uninfected, the virion enters and starts a new cycle of replication; (ii) alternatively, if the cell is already infected then superinfection is blocked, and a new actin tail is formed, propelling the virus further away until it reaches uninfected cells. This mechanism accelerates virus spread and explains how VACV can cross one cell every 1.2 hours as determined by means of live cell imaging. Early expression of proteins A33 and A36 is required, and viruses expressing either protein only late during infection form small plaques. These plaques are closer in size to those formed by the deletion mutants lacking either gene than to wild type, indicating that the formation of actin tails upon superinfection is more important for virus spread than the production of actin tails on cells releasing new virions. All mutations that cause VACV strains to spread poorly and form small plaques also cause dramatic attenuation in vivo, showing the biological importance of rapid spread for VACV virulence (18, 20, 28, 29).

Plaque assays were first described more than 50 years ago (30), and many animal viruses form plaques of size comparable with VACV. Some of these viruses, for instance HSV-1 (31), have replication kinetics similar to VACV, suggesting that other viruses also spread faster than predicted by their replication kinetics. The mechanisms underlying cell-to-cell spread of many viruses remain

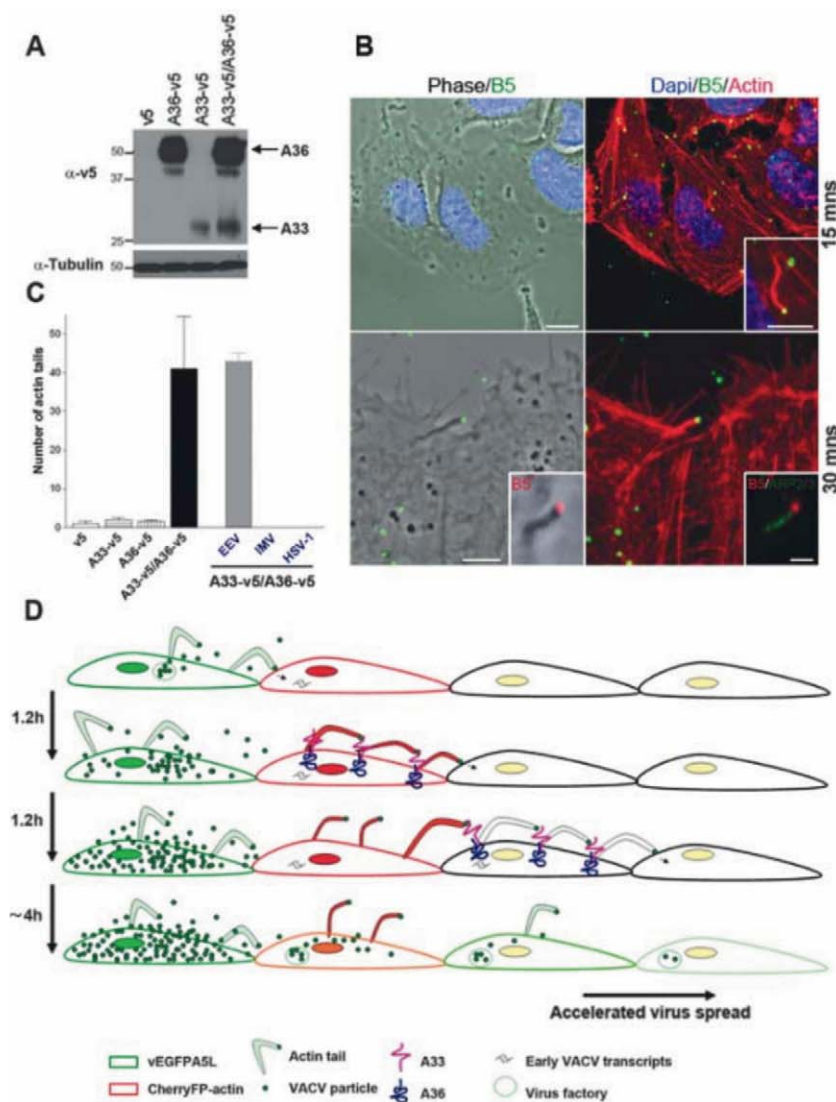


Fig. 4. Expression of A33 and A36 is sufficient for actin tail formation. **(A)** Immunoblot showing A33 and A36 expression. **(B)** Actin tails present 15 and 30 min after spinoculation of EEV particles onto HeLa cells expressing A33 and A36 proteins. Staining for Arp2/3 shows actin polymerisation machinery. Scale bars, top row, 10 μ m; inset, 5 μ m; bottom row, 5 μ m; inset, 1 μ m. **(C)** Graph showing the mean number of actin tails detected per coverslip in the different cell lines. Actin tails were not formed by IMV or HSV-1. Error bars are SEM; $n = 3$ experiments. **(D)** Model showing how VACV spreads rapidly. The first infected cell expresses EGFP-A5 late during infection and releases green virions, which infect an adjacent cell expressing cherryFP-actin (red). Early after infection, A33 and A36 are expressed at the cell surface and mark the cell as infected. Upon contact with new CEV/EEV particles, the A33/A36 complex induces the formation of red actin tails, which repel these virions toward uninfected cells. Superinfecting virions may be repelled from multiple infected cells before an uninfected cell is found that can be infected.

poorly understood, and the elucidation of such mechanisms could lead to the discovery of novel therapeutics.

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Materials and Methods

Figs. S1 to S6

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Movies S1 to S7

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Drive Against Hotspot Motifs in Primates Implicates the *PRDM9* Gene in Meiotic Recombination

Simon Myers,^{1,2*†} Rory Bowden,^{1,2*} Afidalina Tumian,¹ Ronald E. Bontrop,³ Colin Freeman,² Tammie S. MacFie,^{4‡} Gil McVean,^{1,2§} Peter Donnelly^{1,2§}

Although present in both humans and chimpanzees, recombination hotspots, at which meiotic crossover events cluster, differ markedly in their genomic location between the species. We report that a 13–base pair sequence motif previously associated with the activity of 40% of human hotspots does not function in chimpanzees and is being removed by self-destructive drive in the human lineage. Multiple lines of evidence suggest that the rapidly evolving zinc-finger protein *PRDM9* binds to this motif and that sequence changes in the protein may be responsible for hotspot differences between species. The involvement of *PRDM9*, which causes histone H3 lysine 4 trimethylation, implies that there is a common mechanism for recombination hotspots in eukaryotes but raises questions about what forces have driven such rapid change.

In humans and most other eukaryotes, meiotic crossover events typically cluster within narrow regions termed hotspots (1–5). Previously (6), we identified a degenerate 13–base pair (bp) motif, CCNCCNTNNCCNC, that is overrepresented in human hotspots. Both linkage disequilibrium (LD)–based analysis (6) and sperm typing at currently active hotspots (7)

implicated this motif in the activity of 40% of hotspots.

Despite nearly 99% identity at aligned bases, humans and chimpanzees show little if any sharing of hotspot locations (4, 5), although it has remained undetermined whether the recently identified hotspot motif is also active in the chimpanzee. To resolve this question, we collected chimpanzee genetic variation data at 22 loci where there is both an inferred hotspot at the orthologous location in humans and human-chimpanzee sequence conservation of the 13-nucleotide oligomer: 16 motifs within THE1 elements and 6 within L2 elements, chosen for their high activity of a particular “core” version of the motif in humans (fig. S1). We used the statistical software LDhat to estimate recombination rates separately in each region in different populations of both species (8). For humans, we used the Haplotype Map (HapMap) Phase II data. For chimpanzees, we genotyped 36 Western, 20 Central, and 17 Vellorosos chimpanzees at a total of 694 chimpanzee

single-nucleotide polymorphisms (SNPs), an average of 31.5 per region.

Because these regions are inferred human hotspots, the average estimated recombination rate surrounding the motif in humans showed a strong peak for both L2 and THE1 elements (Fig. 1A). In contrast, chimpanzees showed no evidence of increased recombination rates for either background. In Western chimpanzees, the THE1 estimated recombination rate around the motif was similar to the regional average, whereas a weak peak in mean rate for the L2 elements was produced solely by a single potential hotspot in one of the six regions (Fig. 1B). Results for the other chimpanzee subspecies were less informative (fig. S2) (8) but did not reveal a different pattern. To ensure that unknown haplotypic phase, smaller sample size, less dense data, and SNP ascertainment in chimpanzees had not compromised the ability to detect hotspots, we repeatedly sampled from the Centre d'Etude du Polymorphisme Humain (CEPH) from Utah (CEU) HapMap population data to produce human data sets comparable with those from chimpanzees in terms of these features (8). We conditioned only on the presence of the 13-nucleotide oligomer in THE1 and L2 elements and not the presence of a hotspot. This bootstrap technique revealed that the differences between human and chimpanzee rates cannot be explained by differences in power ($P = 0.00052$), although the signal was only significant for THE1 elements when analyzed separately ($P = 0.00012$) (fig. S3). These results provide evidence that the 13-nucleotide oligomer motif does not recruit hotspots in chimpanzees, implying changes in recombination machinery between humans and chimpanzees. The existence of factors capable of such changes in recombination genome-wide has been demonstrated in *Caenorhabditis elegans* (9) and by the mapping in mice of a trans-acting factor responsible for differences in hotspot location among inbred strain crosses (10, 11).

¹Department of Statistics, Oxford University, 1 South Parks Road, Oxford OX1 3TG, UK. ²Wellcome Trust Centre for Human Genetics, Oxford University, Roosevelt Drive, Oxford OX3 7BN, UK. ³Department of Comparative Genetics and Refinement, Biomedical Primate Research Center, Lange Kleiweg 139 2288 GJ, Rijswijk, Netherlands. ⁴Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, UK.

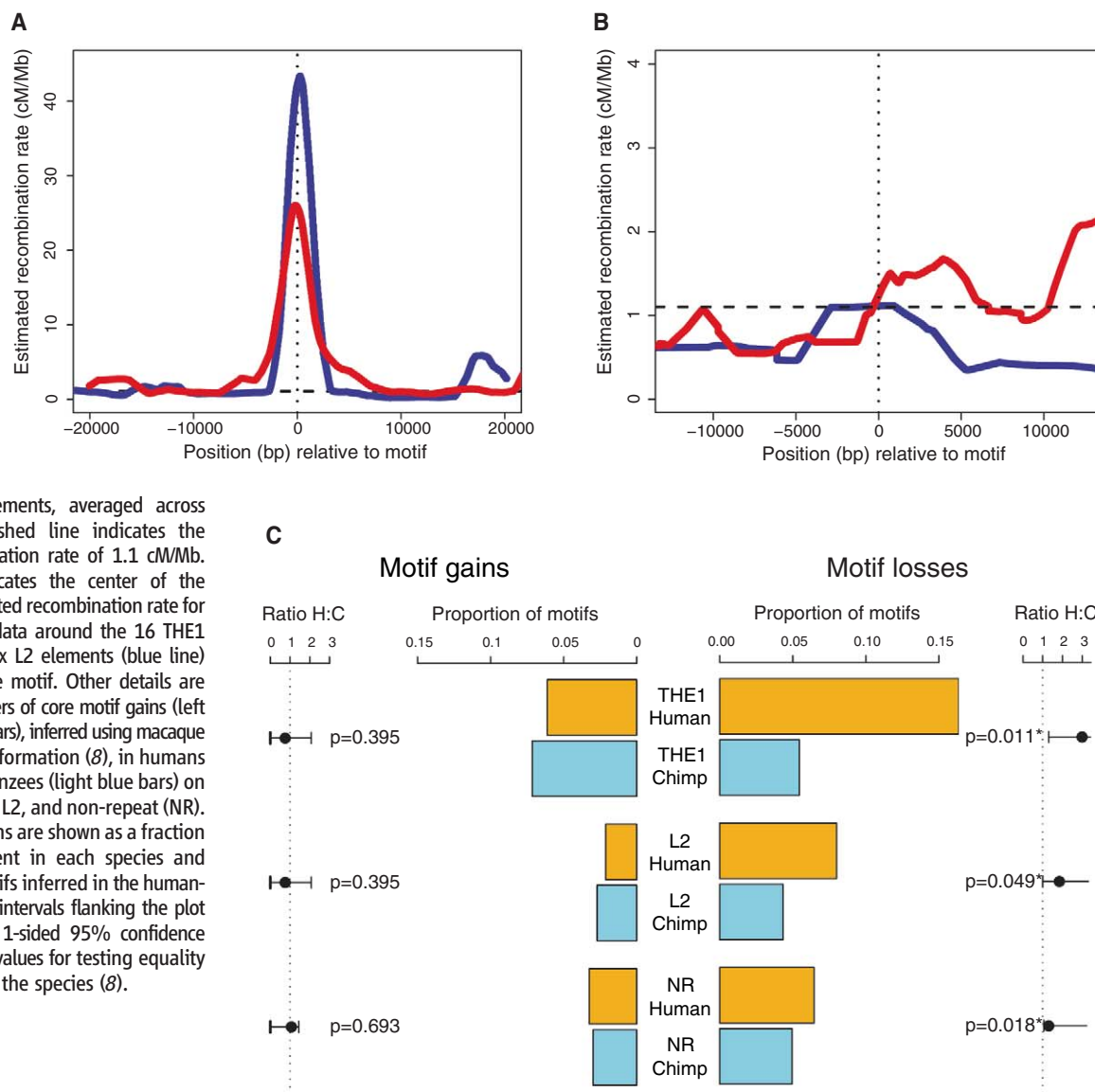
*These authors contributed equally to this work.

†To whom correspondence should be addressed. E-mail: myers@stats.ox.ac.uk

‡Present address: Institute of Cell and Molecular Science, Barts and The London School of Medicine and Dentistry, 4 Newark Street, London E1 2AT, UK.

§These authors contributed equally to this work.

Fig. 1. Recombination rates and patterns of motif gain and loss in human and chimpanzee. For additional details, see (8). (A) Estimated HapMap Phase II recombination rate across the 40 kb surrounding 16 human THE1 elements (red line) and six L2 elements (blue line) orthologous to the 22 regions analyzed in chimpanzee, and each containing a conserved exact match to the 13-bp core motif. Rates are smoothed using a 2-kb sliding window slid in 50-bp increments, averaged across elements. Horizontal dashed line indicates the human average recombination rate of 1.1 cM/Mb. Vertical dotted line indicates the center of the repeat. (B) Average estimated recombination rate for the western chimpanzee data around the 16 THE1 elements (red line) and six L2 elements (blue line) containing the 13-bp core motif. Other details are the same as (A). (C) Numbers of core motif gains (left bars) versus losses (right bars), inferred using macaque and orangutan outgroup information (8), in humans (orange bars) and chimpanzees (light blue bars) on three backgrounds: THE1, L2, and non-repeat (NR). For each background, gains are shown as a fraction of motifs currently present in each species and losses as a fraction of motifs inferred in the human-chimpanzee ancestor. The intervals flanking the plot on each side show exact 1-sided 95% confidence intervals and associated *P* values for testing equality of gain/loss rate between the species (8).



A separate process, predicted to cause a rapid evolution of individual hotspots, is the self-destructive drive inherent in double-strand break (DSB) formation, known as biased gene conversion (BGC) (12). Mutations reducing DSB formation in cis at recombination hotspots are preferentially transmitted as a consequence of repair of DSBs initiated on the other more recombinogenic strand in heterozygotes and are thus favored in a manner mimicking natural selection (13). This phenomenon could lead to rapid hotspot loss (14, 15). Direct evidence from sperm typing (16) has shown BGC at one polymorphic point mutation disrupting an occurrence of the 13-bp motif. More generally, BGC is predicted to eliminate copies of any recombination-promoting motif from the genome. The species-specific recombination activity of the 13-bp human hotspot motif suggests that losses of this motif should have occurred preferentially on the human lineage rather than that leading to chimpanzees.

To examine the evidence for BGC-driven motif loss, we therefore characterized rates and patterns of molecular evolution for the degenerate 13-nucleotide oligomer and the "core" version of the motif on specific backgrounds: THE1 elements, L2 elements, AluY/Sc/Sg elements (degenerate motif only), other repeats, and unique nonrepeat DNA (Table 1). We found a consistent substitution pattern imbalance, with chimpanzees having more copies of the motif than humans [empirical $P = 0.003$ for the most active form, with three of four independent backgrounds showing $P < 0.05$; $P = 0.002$ for the degenerate 13-nucleotide oligomer motif, with $P < 0.05$ for three of five individual backgrounds (8)]. As predicted by theoretical considerations of BGC [supporting online material (SOM) text and table S1] (14, 15), the magnitude of the imbalance was strongest for cases in which the motif has greatest activity. To assess whether motifs have been gained in chimpanzees or lost in humans, we

used the published macaque (17) and draft orangutan (18) genome sequences to infer ancestral sequence. For THE1 elements, L2 elements, and nonrepeat DNA, we observed an excess of human losses of the most active motif relative to chimpanzee ($P < 0.05$ in each case) (Fig. 1C and table S2) and similar results for the degenerate 13-nucleotide oligomer motif (table S3). The effect strength again correlates with hotspot activity. In contrast, there are no significant differences between species in motif gains ($P > 0.3$). Alu elements were not analyzed because of a high rate of uncertainty in inferring the ancestral base.

To determine whether motif activity has been lost on the chimpanzee lineage or gained on the human lineage, we compared our observations with a population-genetics model (SOM text) (14, 15). On the human lineage, approximately 16% of motifs on the THE1 and 8% on the L2 background have been lost in humans since human-chimpanzee divergence (Fig. 1C). If the

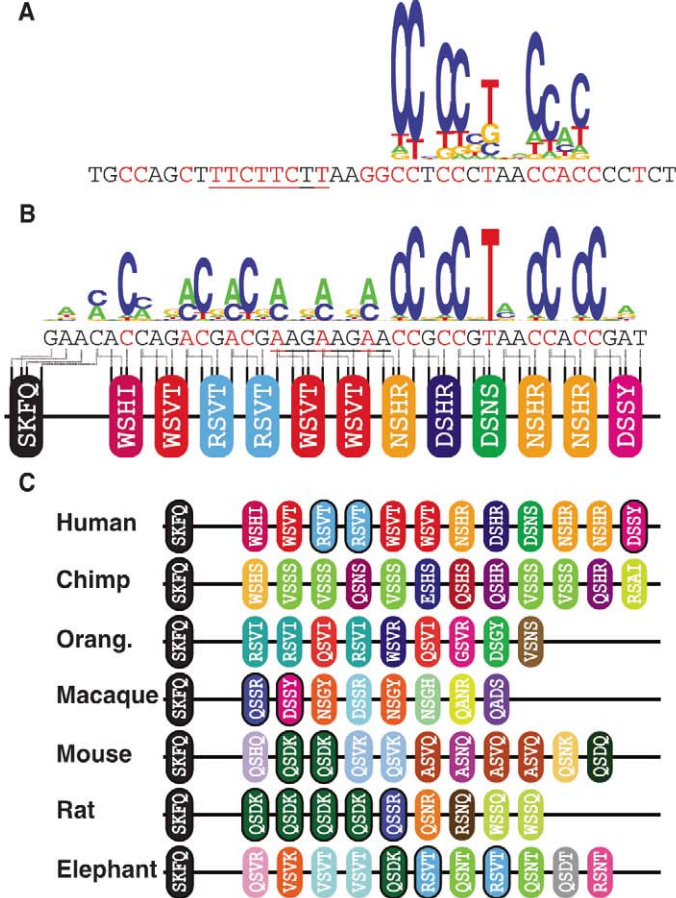
Table 1. Motif imbalance between human and chimpanzee. For the core motif and the degenerate motif, we analyzed cases in which the motif occurs in exactly one of human and chimpanzee. Results are shown for the full set of nonshared motifs and stratified into five backgrounds that differ in average human recombination activity. Significance levels are calculated in two ways: *P* values for

ratios are based on a one-sided exact binomial test of fewer human-only cases because the motif is known to be active in humans. Empirical *P* values are one-sided and obtained through comparisons of counts for the core or degenerate motif with counts observed for motifs of the same length and GC content on the same backgrounds (8). Dashes indicate zero counts in both species.

Sequence background	Core motif CCTCCCTNNCCAC				Degenerate motif CCNCCNTNNCCNC			
	Human only	Chimp only	Ratio (<i>P</i> value)	Empirical <i>P</i> value	Human only	Chimp only	Ratio (<i>P</i> value)	Empirical <i>P</i> value
All	425	515	1.21 (0.0018**)	0.0033**	19448	20245	1.04 (3.2e-05**)	0.0020**
THE1	20	39	1.95 (0.0092**)	0.0050**	50	76	1.52 (0.0128*)	0.0093**
L2	30	47	1.57 (0.0338*)	0.0307*	432	496	1.15 (0.0193*)	0.0219*
AluY,Sc,Sg	—	—	—	—	3642	3924	1.08 (0.0006**)	0.1119
Other repeats	99	131	1.32 (0.0204*)	0.0346*	10126	10254	1.01 (0.1868)	0.4373
Non-repeats	276	298	1.08 (0.2135)	0.2206	5198	5495	1.06 (0.0021**)	0.0215*

P* < 0.05. *P* < 0.01.

Fig. 2. (A) Previously estimated degeneracy of the 13-bp hotspot motif (logo plot; relative letter height proportional to estimated probability of hotspot activity and total letter height determined by degree of base specificity) (6) as well as an extended ~39-bp motif [text below logo, with influential positions (*P* < 0.01) shown in red]. (B) In silico prediction of the binding consensus for PRDM9, aligned with the 13-nucleotide oligomer, with more influential positions shown in red. Underlined in both (A) and (B) is an additional 8-bp matching sequence. The logo shows predicted degeneracy within this consensus (8). Below the text is the sequence of four predicted DNA-contacting amino acids for the 13 successive human PRDM9 zinc fingers (one oval per finger, differing colors for differing fingers, and the separated finger is gapped N-terminal from others) and their predicted base contacts within the motif. (C) Sequence of four predicted DNA-contacting amino acids for the PRDM9 zinc fingers in seven mammalian species, presented as in (B). Distinct fingers are given different colors; fingers present in at least two species have a black border.



zinc-finger proteins, the 13-nucleotide oligomer motif was present within the predicted binding sequence of five (fig. S4). Binding specificity was then further explored in silico by comparing predicted motif degeneracy for each candidate (inferred by calculating the relative binding score for every 1-bp mutation relative to the consensus) with empirical degeneracy patterns in the 13-bp motif (Fig. 2A). Predictions for one of the candidates, PRDM9, exactly matched the observed degeneracy at positions 3, 6, 8, 9, and 12 within the 13-bp motif (Fig. 2B) and lack of degeneracy at the other eight positions. Predictions for the other four candidates showed features inconsistent with the observed degeneracy (fig. S4). The predicted binding sequence for PRDM9 also contains an exact match on the opposite strand for an 8-bp region of the extended motif, upstream of the 13-bp degenerate motif, perhaps suggesting that PRDM9 zinc fingers might contact both DNA strands. Finally, the number of zinc fingers (13) in this protein, the positioning of the match to the 13-bp motif within the longer predicted binding sequence, and strong influence of this 13-bp region on specificity all match our previous predictions (6). The lack of activity of the 13-bp motif in chimpanzees demonstrated above suggests that in addition to having the predicted binding specificity, any motif-binding protein candidate should also show differences between humans and chimpanzees. For four of the five candidates, the predicted DNA-contacting amino acids within the zinc fingers are identical between human and chimpanzee. Chimpanzee PRDM9, however, has a dramatically different predicted binding sequence (fig. S5). Although PRDM9 has multiple zinc fingers in both species (12 and 13 respectively), the DNA-contacting residues -1, 2, 3, and 6 are only shared between species in the first finger (Fig. 2C). Such rapid evolution is exceptional. Comparing these residues among all 544 C2H2-containing zinc-finger protein human-chimpanzee ortholog pairs, PRDM9 is the most diverged (*P* = 0.0018). The PRDM9 sequences in five additional mammals (elephant, mouse, rat, macaque and orangutan) exhibit rapid evolution, variation in zinc-finger number (between 8 and

12), and patterns of substitution suggestive of complex repeat shuffling (Fig. 2C) (20).

Multiple lines of evidence point to a role for the orthologous mouse gene, *Prdm9*, in recombination. *Prdm9* lies within a 5.1-Mb region that contains a locus that influences genome-wide hotspot locations (10, 11) and is exclusively expressed during meiotic prophase, with mice in which *Prdm9* has been knocked out showing infertility and failure to properly repair DSBs (21). Mouse PRDM9 trimethylates lysine 4 of histone H3 (H3K4me3) (21), an epigenetic mark specifically enriched on mouse chromatids carrying recombination initiation sites within the mouse hotspot *Psb9* (22). In yeast, mutation of the sole gene, *Set1*, encoding H3K4me3 reduces crossover activity at 84% of hotspots (23). The lack of well-defined target-sequence specificity of Set1 (which is not a zinc-finger protein) may indicate why no dominant hotspot motif has been identified in yeast. Intriguingly, *Prdm9* is also the only species-incompatibility gene yet identified in mouse (24), with differences among nine PRDM9 zinc fingers between mouse strains potentially playing a causal role in male sterility.

Baudat *et al.* find that variation in *PRDM9* among humans correlates with variability in genome-wide hotspot usage, and PRDM9 binds the 13-bp motif in a sequence-specific manner in vitro (25). The findings of both studies imply that PRDM9 determines human hotspot locations, with PRDM9 evolution explaining lack of hotspot conservation in other species. Exactly how PRDM9 functions, for example through altering transcription of DSB repair genes or directly recruiting DSB repair proteins, remains unknown. These findings also raise the question of why such an important gene is evolving so rapidly. The

DNA sequence of the zinc-finger array of PRDM9 constitutes a coding minisatellite, suggesting a high intrinsic mutation rate resulting from repeat instability. However, patterns of evolution within the zinc-finger array, notably the clustering and coordination of changes at sites that interact with DNA bases, strongly suggest positive selection on binding specificity (20). Selection could possibly arise from the gradual degradation of hotspots through BGC, leading to a loss in fitness either through the promotion of deleterious alleles within hotspots (15) or through having insufficient crossover events to support proper disjunction (14, 15). Alternatively, the rapid evolution of *PRDM9* could be indicative of genetic conflict, such as meiotic drive or conflict involving mobile elements (26, 27). Although there is no direct evidence for this, mouse *Prdm9* lies within one of the inversions characterizing the meiotic-drive *t*-complex (28).

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Supporting Online Material

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Materials and Methods
SOM Text
Figs. S1 to S5
Tables S1 and S2
References
Database S1

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The *Lmo2* Oncogene Initiates Leukemia in Mice by Inducing Thymocyte Self-Renewal

Matthew P. McCormack,^{1,2*} Lauren F. Young,¹ Sumitha Vasudevan,¹ Carolyn A. de Graaf,³ Rosalind Codrington,⁴ Terence H. Rabbitts,⁴ Stephen M. Jane,^{1,2} David J. Curtis^{1,2}

The *LMO2* oncogene causes a subset of human T cell acute lymphoblastic leukemias (T-ALL), including four cases that arose as adverse events in gene therapy trials. To investigate the cellular origin of *LMO2*-induced leukemia, we used cell fate mapping to study mice in which the *Lmo2* gene was constitutively expressed in the thymus. *Lmo2* induced self-renewal of committed T cells in the mice more than 8 months before the development of overt T-ALL. These self-renewing cells retained the capacity for T cell differentiation but expressed several genes typical of hematopoietic stem cells (HSCs), suggesting that *Lmo2* might reactivate an HSC-specific transcriptional program. Forced expression of one such gene, *Hhex*, was sufficient to initiate self-renewal of thymocytes in vivo. Thus, *Lmo2* promotes the self-renewal of preleukemic thymocytes, providing a mechanism by which committed T cells can then accumulate additional genetic mutations required for leukemic transformation.

Despite significant improvements in the treatment of T cell acute lymphoblastic leukemia (T-ALL), about 20% of chil-

dren and the majority of adults succumb to resistant or relapsed disease (1). A common cause of T-ALL is the overexpression of onco-

genic transcription factors during T cell development in the thymus, resulting from chromosomal translocations or deletions (2). One such oncogene is *LMO2*, whose expression is activated in about 9% of pediatric T-ALL cases (3). *LMO2* is a member of the LMO (LIM-only) class of transcription factors that contain zinc-binding finger-like motifs termed LIM domains, which function in protein-protein interactions (4). *Lmo2* does not bind DNA directly but mediates transcriptional activation and repression by binding to other transcription factors to mediate the formation of multimeric complexes (5).

¹Rotary Bone Marrow Research Laboratories, Royal Melbourne Hospital, Grattan Street, Parkville, Victoria 3050, Australia.

²Department of Medicine, University of Melbourne, Parkville, Victoria 3010, Australia. ³The Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville, Victoria 3050, Australia, and Department of Medical Biology, University of Melbourne, Parkville, Victoria 3010, Australia. ⁴Leeds Institute of Molecular Medicine, Wellcome Trust Brenner Building, St. James's University Hospital, Leeds LS9 7TF, UK.

*To whom correspondence should be addressed. E-mail mccormack@wehi.edu.au

In addition to its involvement in pediatric T-ALL, LMO2 expression was activated by retroviral insertion mutagenesis in four patients receiving gene therapy for X-linked severe combined immunodeficiency (SCID-X1), resulting in leukemia (6–9). In these patients, leukemia occurred long after therapy (31 months on average) and contained multiple additional genetic abnormalities. To permit such clonal selection, the cell of origin must possess the property of self-renewal, which is normally reserved for hematopoietic stem cells (HSCs) within the bone marrow (BM).

To investigate the cellular origin of T-ALL, we applied a cell fate mapping strategy to a mouse model of T-ALL to determine when the leukemia-initiating cell (LIC) is established within the thymus. *CD2-Lmo2* transgenic mice express *Lmo2* in the thymus and develop a T cell leukemia similar to human T-ALL, after a mean latency of 10 months (10). During this period, the animals display a preleukemic phenotype characterized by an accumulation of immature, double negative (DN) thymocytes that do not express the lineage markers CD4 and CD8 (11). We intercrossed *CD2-Lmo2* transgenic mice with *Mx1-Cre* transgenic and

Rosa26-YFP (YFP is yellow fluorescent protein) Cre-reporter mice to generate *Lmo2;Mx;YFP* triple transgenic mice (12). Injection of polyinosinic-polycytidylic acid (PI-PC) into mice expressing the *Mx1-Cre* transgene leads to efficient deletion of *loxP*-flanked sequences in HSCs but not thymocytes (13). This property allowed us to selectively mark BM cells and track their migration to the thymus. *Lmo2;Mx;YFP* triple-transgenic mice (along with *Mx;YFP* controls) were injected with PI-PC at 7 weeks of age, and the expression of YFP in BM progenitors and thymocytes was analyzed subsequently (Fig. 1A). Seven days after injection, 90% of BM progenitors (lineage-negative, Kit+) from control *Mx;YFP* animals were YFP-positive, compared with only 15% of thymocytes. Consistent with normal thymic turnover from BM progenitors (14), >90% of thymocytes became YFP-positive by 1 month after PI-PC treatment. In contrast, although *Lmo2;Mx;YFP* mice displayed normal induction of YFP expression in BM progenitors, there was no increase in YFP-positive cells in the thymus, indicating that thymic import from the BM was completely blocked. Despite this, the thymi of *Lmo2*-transgenic mice were only slightly reduced in

cellularity (fig. S1A) and had a polyclonal T cell repertoire, as determined by T cell receptor (TCR) V β -subfamily usage (fig. S1B). Similar results were obtained in 10-day-old mice injected with PI-PC (fig. S1C). Importantly, T cell leukemias, which developed in *Lmo2;Mx;YFP* mice an average of 8 months after PI-PC injection, were always YFP-negative (Fig. 1B). These results indicate that the LIC is established within the thymus of *Lmo2*-transgenic mice at least 8 months before the development of leukemia.

To determine whether thymic turnover in *Lmo2*-transgenic mice could be restored by using radiation therapy, we exposed PI-PC-treated *Lmo2;Mx;YFP* mice to sublethal irradiation [6.5 grays (Gy)] to kill the majority of thymocytes. Two days after irradiation, 99% of both control and *Lmo2*-transgenic thymocytes were eliminated (Fig. 1C). After a 3-week recovery period, *Lmo2*-transgenic thymocytes had expanded normally and contained all T cell subsets (Fig. 1C). Despite this, they remained YFP-negative, indicating that thymic regeneration was derived from radio-resistant thymocytes rather than BM-derived progenitors (Fig. 1D). This thymic regeneration was polyclonal, suggesting that it was not due to

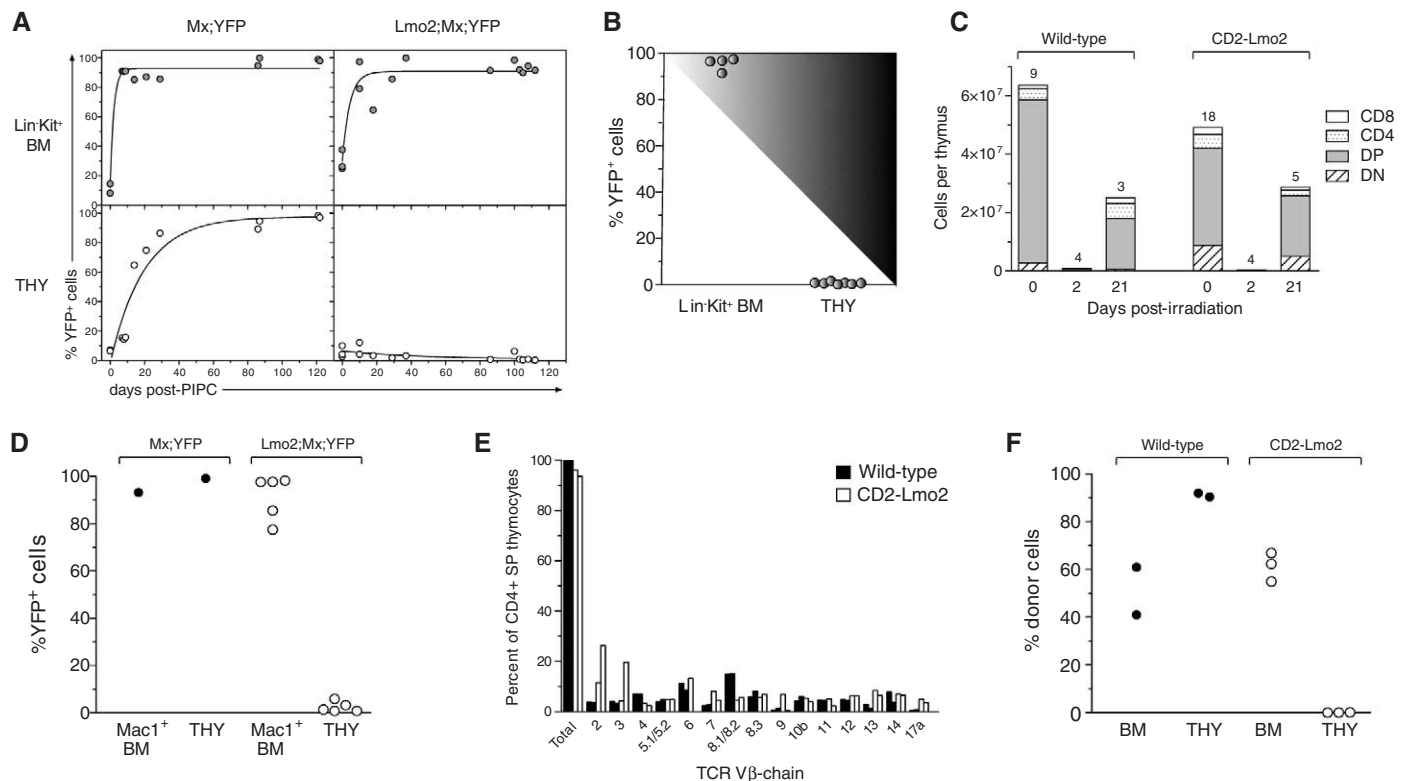


Fig. 1. *Lmo2*-induced T cell malignancy has an early thymic origin. (A) *Lmo2* blocks thymic turnover. Mice were injected with PI-PC at 7 weeks of age, and then the percentage of YFP-positive BM progenitors (Lin^{Kit}⁺ BM) and thymocytes (THY) was determined by flow cytometry. (B) LICs in *Lmo2* transgenic mice are resident in the thymus before 7 weeks of age. *Lmo2;Mx;YFP* mice were treated with PI-PC as above. When leukemia was clinically evident, the percentage of YFP-positive BM progenitors and thymocytes was determined. (C) Response of *Lmo2*-transgenic mice to irradiation. Preleukemic *Lmo2*-transgenic mice were irradiated (6.5 Gy) at day 0, and the immunophenotype of various

thymic populations was determined. Numbers indicate the mice used per experiment. (D) Blocked thymic turnover in *Lmo2* transgenic mice after irradiation. Mice were irradiated (6.5 Gy), and the proportions of YFP-positive BM myeloid cells (Mac1⁺ BM) and thymocytes (THY) were determined after 3 weeks. (E) Regenerated thymi in *Lmo2* transgenic mice are polyclonal. Three weeks after irradiation, TCR V β -subfamily usage was determined for thymic CD4⁺ SP thymocytes. Bars denote data from individual mice. (F) Mice were irradiated (6.5 Gy) and injected with 10⁷ Ly5.1 BM cells. The extent of donor contribution to the BM and thymus (THY) was determined after 3 weeks.

the outgrowth of a rare clone of radioresistant thymocytes (Fig. 1E). Furthermore, injection of wild-type BM cells into irradiated *Lmo2*-transgenic mice led to effective repopulation of the BM but not the thymus (Fig. 1F), indicating that radioresistant *Lmo2*-transgenic thymocytes can outcompete nonirradiated BM-derived progenitors.

To explain these findings, we hypothesized that a fraction of preleukemic *Lmo2* transgenic thymocytes have stem cell-like properties, namely, long-term self-renewal and the ability to generate differentiated cells. To confirm this, we transplanted 2.5×10^7 thymocytes from 8-week-old *Lmo2*-transgenic mice into sublethally irradiated Ly5.1 congenic recipients (Fig. 2, A and B). Three weeks after transplantation, wild-type thymocytes gave rise only to small numbers ($0.75 \pm 0.78 \times 10^6$, $n = 6$) of mature, CD4- and CD8-single-positive (SP) T cells. In contrast, *Lmo2*-transgenic thymocytes

gave robust engraftment (43 ± 5.0 million, $n = 12$) of all T cell subsets [DN, CD4⁺CD8⁺ double positive (DP), and SP cells]. Hence, *Lmo2* confers on thymocytes the stem cell-like property of stable engraftment.

To determine which thymocyte subsets had engraftment potential, we transplanted fluorescence-activated cell sorting (FACS)-isolated DN thymocytes or a combination of more mature DP and SP thymocytes. This demonstrated that long-term repopulating ability was restricted to DN thymocytes (Fig. 2C). Upon further fractionation, this property was additionally restricted to cells of the DN3 subset (Fig. 2, D and E), which expressed multiple T cell differentiation markers (CD2, CD5, and Thy1.2), confirming that they were committed T cells (Fig. 2, F to H).

A hallmark of self-renewing stem cells is that they persist in serial transplantation exper-

iments. Accordingly, *Lmo2*-transgenic thymocytes were capable of repopulating the thymus in secondary, tertiary, and quaternary recipients (Fig. 2I), while retaining a capacity to differentiate into DP and SP thymocytes (Fig. 2J). These serially transplanted thymocytes could give rise to peripheral T cells but not B cells or myeloid cells (fig. S2A). However, even after quaternary transplant, long-term engraftment capacity remained restricted to DN thymocytes (fig. S2B). Thus, *Lmo2* induces self-renewal of committed T cells while maintaining T cell-restricted differentiation potential. In contrast, mice transgenic for the T cell oncogene *Notch3* did not show demonstrable thymocyte self-renewal, suggesting that this is not a general feature of T cell oncogenes (fig. S3).

Because *Lmo2* is normally required for development of HSCs but not T cells, we

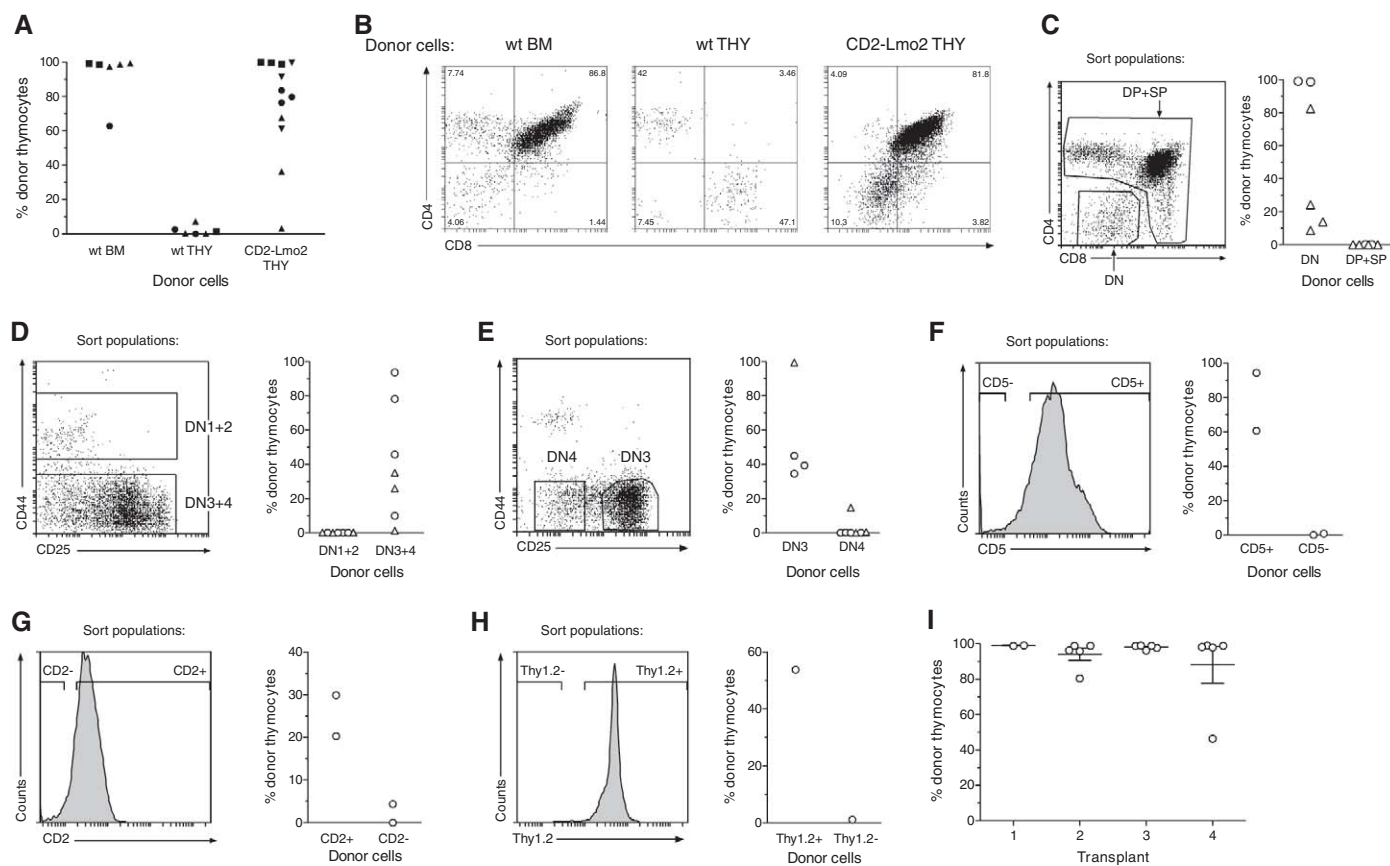


Fig. 2. *Lmo2* induces self-renewal of thymocytes. **(A)** *Lmo2*-transgenic thymocytes have long-term engraftment potential. We injected 2.5×10^7 cells from wild-type (wt) BM, wt thymus, or *CD2-Lmo2* transgenic thymus into irradiated (6.5 Gy) Ly5.1 recipients. Three weeks later, the percentage of donor thymocytes was determined. Points represent individual recipient mice, and unique symbols denote separate experiments. **(B)** Representative CD4/CD8 profiles of donor thymocytes 3 weeks after transplant. **(C to H)** Long-term engraftment potential is restricted to DN3 thymocytes. Total CD45⁺ [(C) and (F) to (H)] or DN-gated [(D) and (E)] *Lmo2*-transgenic thymocytes were fractionated, and cells from one-quarter of a thymus were injected into irradiated (6.5 Gy) Ly5.1 recipients. Three weeks later, the percentage of donor thymocytes was determined. Negative gating regions were set by using isotype control antibodies. **(I)** *Lmo2* induces self-renewal

of thymocytes. DN thymocytes from *Lmo2*-transgenic mice were injected into irradiated (6.5 Gy) Ly5.1 recipients. Cells from one-quarter of a thymus were then serially transplanted at 3-week intervals. Bars represent mean $\pm$ SEM. **(J)** Representative CD4/CD8 profiles of thymocytes from recipient mice after serial transplantation. Numbers indicate the percent of cells in each quadrant.

postulated that it might promote self-renewal of thymocytes by reactivation of an HSC-specific transcriptional program (15–18). To examine this, we isolated DN thymocytes from wild-type and *Lmo2*-transgenic mice and compared their gene expression profiles using microarrays. Remarkably, only 113 genes were differentially expressed between the two populations (81 increased and 32 reduced), indicating that preleukemic thymocytes in *Lmo2*-transgenic mice are similar to their wild-type counterparts (tables S1 and S2). The majority of *Lmo2*-up-regulated genes were more highly expressed in normal HSC-enriched lineage-negative Kit⁺ Sca⁺ (LKS) cells than in normal DN T cells, implying that these genes are normally down-regulated during early T cell development (fig. S4A). In contrast, genes down-regulated in *Lmo2*-transgenic DN thymocytes showed significant association with normal DN T cells compared with LKS cells (fig. S4B). These included several genes involved in T cell differentiation, such as the pre-TCR α (*Ptcr*) and TCR β (*Tcrb*) subunit genes. Hence, expression of *Lmo2* in thymocytes causes up-regulation of several HSC-associated genes in addition to repression of T cell developmental genes.

We next examined the relative expression of *Lmo2*-up-regulated genes in a data set of 27 human T-ALL samples, including three distinct sub-

classes expressing the transcription factors *LYL1*, *HOX11*, or *TAL1* (19). In this study, *LMO2* expression was associated with the *LYL1* subset of T-ALL. Accordingly, a significant association was observed between the mouse *Lmo2*-induced expression profile and *LYL1*-associated T-ALL cases (Fig. 3A), suggesting that the signatures in human T-ALL are relevant to their initiating events.

The transcripts of several *Lmo2*-induced genes were enriched in both normal LKS cells and *LYL1*-associated T-ALLs, including *Lyl1*, *Hhex*, *Kit*, and *Nfe2* (fig. S4A and Fig. 3A). We examined three genes with known roles in HSC function, namely *Hhex*, *Kit*, and *Lyl1* (along with *Lmo2*), by expressing them in the mouse hematopoietic system using a BM reconstitution model. Retrovirus-mediated expression of *Lmo2* induced long-term engraftment of thymocytes in secondary recipient mice, consistent with our earlier findings (Fig. 3B). Moreover, overexpression of the homeobox transcription factor *Hhex*, but not *Kit* or *Lyl1*, also induced long-term engraftment of thymocytes, indicating that expression of *Hhex* is sufficient to induce thymocyte self-renewal (Fig. 3B). Both *Lmo2*- and *Hhex*-overexpressing thymocytes contained an abnormal cell population that expressed *Kit*, *CD25*, and *CD5*, suggesting that *Lmo2* and *Hhex* induce self-renewal of a similar

cell type (fig. S5). Thus, overexpression of *Lmo2* in thymocytes induces the expression of a homeobox transcription factor, *Hhex*, which can initiate thymocyte self-renewal.

Our data suggest that long-term thymocyte self-renewal is the cause of T cell leukemia in *Lmo2*-transgenic mice. Thymocyte self-renewal provides a mechanism for committed T cells to accumulate additional genetic mutations required for leukemic transformation. This could explain the selective advantage of T cells with retroviral insertional activation of *LMO2* after gene therapy for SCID-X1 as well as the detection of proviruses adjacent to the *LMO2* locus up to 21 months before the development of T-ALL in three of four patients (6, 8, 9).

The heterogeneity and multigenic nature of leukemia lead to inherent difficulties in the identification of critical genetic pathways and therapeutics that can target the LIC. For example, drugs may target proliferative effects due to secondary mutations without killing the LIC and therefore fail to prevent relapse. In keeping with this, we have shown that, in *Lmo2*-transgenic mice, LICs survive radiation therapy despite over 99% killing of thymocytes. These mice may be a useful tool for identifying therapeutics that target the self-renewal capacity of LICs.

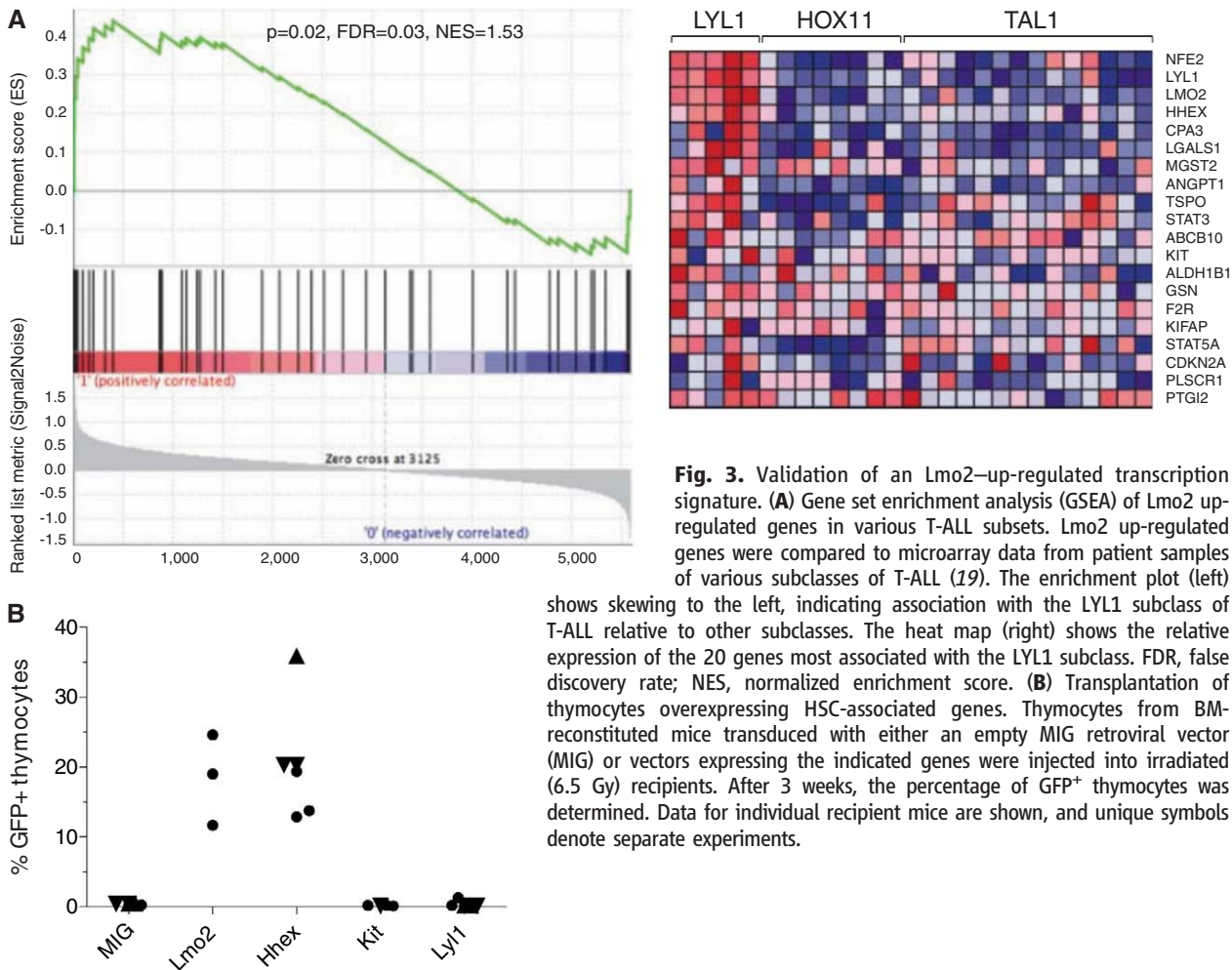


Fig. 3. Validation of an *Lmo2*-up-regulated transcription signature. **(A)** Gene set enrichment analysis (GSEA) of *Lmo2* up-regulated genes in various T-ALL subsets. *Lmo2* up-regulated genes were compared to microarray data from patient samples of various subclasses of T-ALL (19). The enrichment plot (left) shows skewing to the left, indicating association with the *LYL1* subclass of T-ALL relative to other subclasses. The heat map (right) shows the relative expression of the 20 genes most associated with the *LYL1* subclass. FDR, false discovery rate; NES, normalized enrichment score. **(B)** Transplantation of thymocytes overexpressing HSC-associated genes. Thymocytes from BM-reconstituted mice transduced with either an empty MIG retroviral vector (MIG) or vectors expressing the indicated genes were injected into irradiated (6.5 Gy) recipients. After 3 weeks, the percentage of GFP⁺ thymocytes was determined. Data for individual recipient mice are shown, and unique symbols denote separate experiments.

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Figs. S1 to S5
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A Composite of Multiple Signals Distinguishes Causal Variants in Regions of Positive Selection

Sharon R. Grossman,^{1,2*} Ilya Shylakhter,^{1,2*} Elinor K. Karlsson,^{1,2} Elizabeth H. Byrne,^{1,2} Shannon Morales,^{1,2,3} Gabriel Frieden,¹ Elizabeth Hostetter,^{1,2} Elaine Angelino,^{1,4} Manuel Garber,² Or Zuk,² Eric S. Lander,^{2,4,5} Stephen F. Schaffner,² Pardis C. Sabeti^{1,2,4†}

The human genome contains hundreds of regions whose patterns of genetic variation indicate recent positive natural selection, yet for most the underlying gene and the advantageous mutation remain unknown. We developed a method, composite of multiple signals (CMS), that combines tests for multiple signals of selection and increases resolution by up to 100-fold. By applying CMS to candidate regions from the International Haplotype Map, we localized population-specific selective signals to 55 kilobases (median), identifying known and novel causal variants. CMS can not just identify individual loci but implicates precise variants selected by evolution.

Numerous methods have been developed to exploit signatures left by positive natural selection to identify genomic regions in the human genome harboring recent local adaptations, presumably to such pressures as infectious disease, changes in diet, and new environments (1, 2). Hundreds of such regions have been identified, but they are typically large (hundreds of kilobases to megabases) and contain many genes and thousands of polymorphisms. In only a handful has there been much progress in identifying the causal mutations and extracting these biological insights about their function. More powerful methods are needed to pinpoint the exact mutations driving evolution, especially as increasingly powerful sequencing technologies make it possible to sequence the genomes of humans and many other species.

Initial surveys of selective events have relied on three patterns of variation caused by a new beneficial mutation rising quickly in prevalence in a population: (i) Long haplotypes: An allele under positive selection increases in frequency so rapidly that long-range associations with neighboring polymorphisms—the “long-range haplotype”—are not disrupted by recombination. (ii) High-frequency derived alleles: A new (nonancestral, or derived) allele rises to a frequency higher than expected under genetic drift, carrying neighboring derived alleles with it. (iii) Highly differentiated alleles: Positive selection in one geographic region causes larger frequency differences between populations than for neutrally evolving alleles. In humans, these three signals are detectable back to between 30,000 to 80,000 years ago (2).

If each signature provides distinct information about selective sweeps, combining the signals should have greater power for localizing the source of selection than any single test. As inputs to a composite statistic we chose two established metrics for haplotype length (iHS and XP-EHH) (3, 4) and one for population differentiation (F_{ST}) (5). We also developed and incorporated two additional tests. Δ DAF tests for derived alleles that are at high frequency relative to other populations; it is more sensitive for distinguishing

selected alleles than the simple derived allele frequency (DAF, fig. S1). Δ iHH measures the absolute rather than the relative length of haplotypes and is particularly sensitive for identifying lower-frequency selected alleles.

To characterize each test's ability to localize signals of recent local adaptation spatially and to distinguish causal variants from nearby neutral markers, we simulated neutrally evolving regions and regions containing a positively selected allele by standard coalescent approaches (6). We tested a range of demographic models, including a standard neutral model; a calibrated model of European, East Asian, and West African populations; and several more extreme models. Regions under selection were modeled as containing a single, centrally located selected variant that appeared within the last 5000 to 30,000 years, was subject to a specified intensity of selection, and rose to present-day frequencies ranging from 20 to 100% (table S1).

For each model set we generated 1500 replicates, each consisting of 1 Mb of simulated sequence data (~10,000 polymorphisms) for 120 chromosomes from each population. In addition, we generated a data set that matched the frequency distribution and density of Phase II of the International Haplotype Map Project (HapMapII) (7).

Under all scenarios, each of the five statistics had distinguishable distributions for causal and for neutral variants (including neutral variants in selected regions). The F_{ST} and XP-EHH signals peaked more narrowly around the causal variant, making them useful for spatial localization, but poorly distinguished the precise causal variant (Fig. 1 and fig. S2). In contrast, iHS, Δ iHH, and Δ DAF contributed little to spatial resolution, but better distinguished causal variants. The five tests were nearly uncorrelated in neutral regions, and only weakly correlated for neutral variants within selected regions (fig. S3). In the latter case, correlation was appreciable only immediately around the causal variant.

As each of the five tests had power to distinguish selected from nonselected variants and were only weakly correlated for neutral variants, we combined them in a composite likelihood statistic, termed the composite of multiple signals

¹Center for Systems Biology, Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02138, USA. ²Broad Institute of MIT and Harvard, Cambridge, MA 02142, USA. ³Mount Sinai School of Medicine, New York, NY 10029, USA. ⁴Department of Systems Biology, Harvard Medical School, Boston, MA 02115, USA. ⁵Department of Biology, MIT, Cambridge, MA 02139, USA.

*These authors contributed equally to this work.

†To whom correspondence should be addressed. E-mail: psabeti@oeb.harvard.edu (P.C.S.), shari.grossman@post.harvard.edu (S.R.G.), ilya_shl@alum.mit.edu (I.S.)

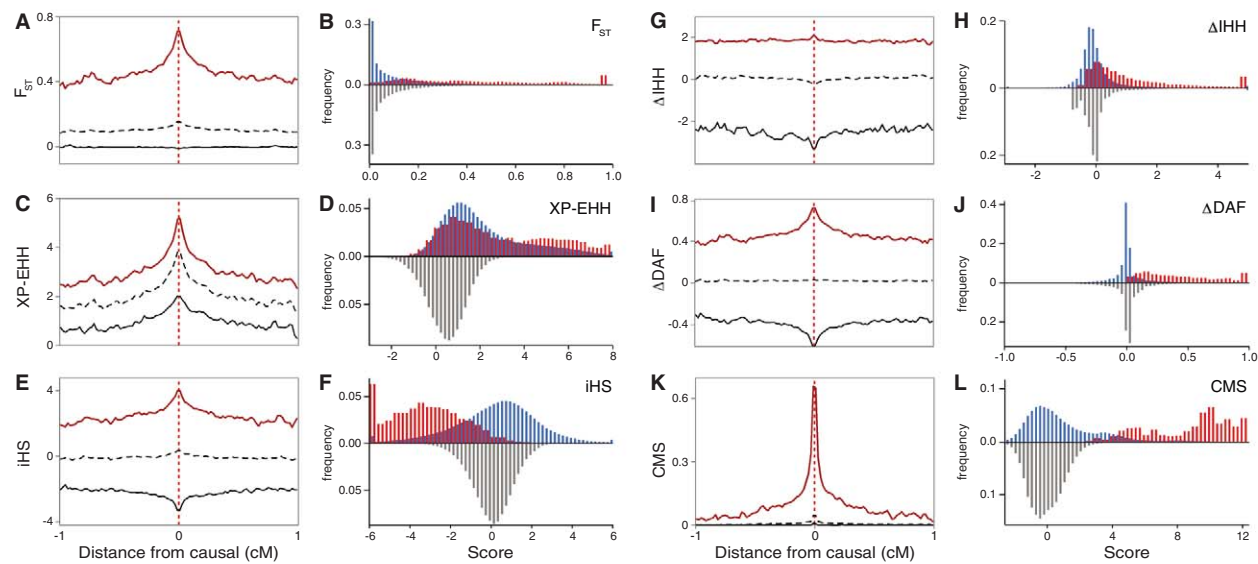


Fig. 1. CMS localizes selection and identifies causal variants better than single tests. (A, C, E, G, I) Top 5% (red line) and bottom 5% (black line) of scores and mean score (black, dashed line) in 1 MB surrounding causal mutation (located at red dashed line). (B, D, F, H, J) Distribution of

scores for the causal variant (red bars), nearby unselected variants (blue bars), and variants in regions without selection (gray bars, below axis). The composite test (CMS) outperforms individual tests for (K) localizing the selective signal and (L) distinguishing the causal variant.

(CMS). For each test i , we estimated from simulation the probability P of a score s_i if selected and if unselected. Assuming a uniform prior probability of selection π , the CMS score is the approximate posterior probability that the variant is selected:

$$CMS = \prod_{i=1}^n \frac{P(s_i|selected) \times \pi}{P(s_i|selected) \times \pi + P(s_i|unselected) \times (1 - \pi)} \quad (1)$$

We calculate the CMS score and significance (on the basis of the genome-wide distribution of scores) for every variant. To localize a signal, the distribution of CMS scores across the entire region is used to estimate a posterior probability curve for the position of the causal variant and determine 90% credible intervals [supporting on-line material (SOM)].

In simulations, CMS showed power both to localize the selection signal spatially and distinguish the causal variant (Fig. 1, K and L). Whereas single tests provided weak localization (~1 Mb), CMS localized the signal to an average 89 kb (for full sequence data) and contained the causal variant in 90% of cases. With sparser genotype data (corresponding to HapMapII), CMS localized to 104 kb, even when the causal variant was absent from the data set. CMS also showed greater specificity for the causal variant. At score thresholds giving 90% power to detect the true causal variant, the individual tests identified ~500 to 1500 candidate causal variants per region, whereas CMS narrowed the signal to ~100 (table S2). The causal variant was among the top 20 variants in half of cases and was the highest-scoring variant in a quarter of cases, with high power given that we included sweeps to frequencies as low as 20%. The power for

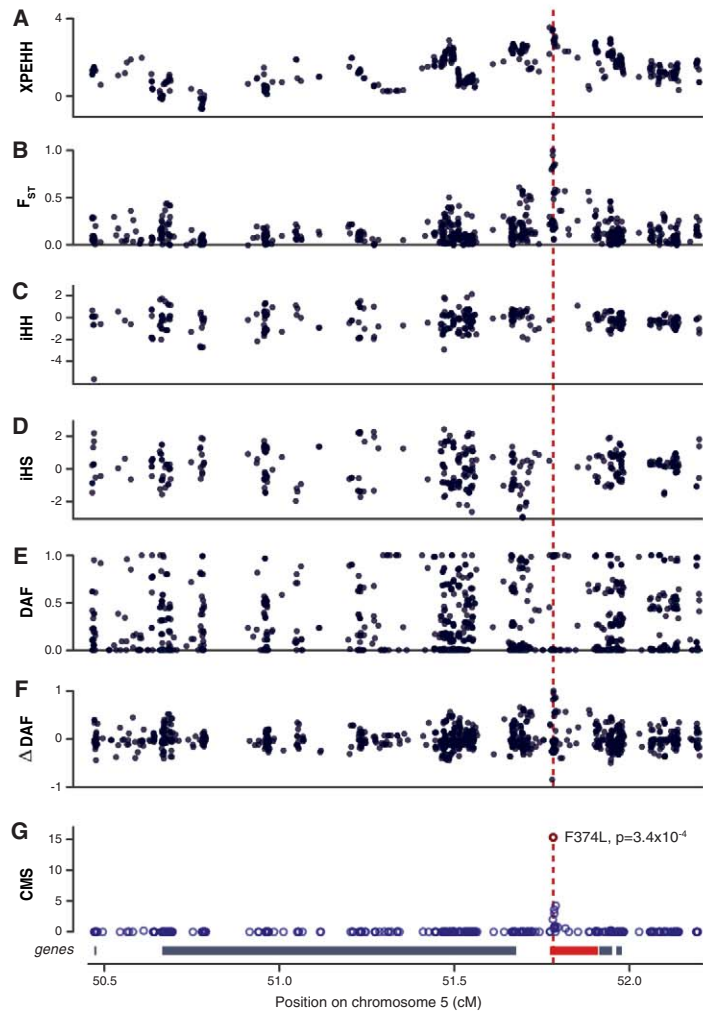


Fig. 2. Localizing selection at *MATP*. Scores of six individual tests (A to F) and CMS (G) for a region containing *MATP*. A nonsynonymous SNP [rs16891982, F374L (Phe³⁷⁴→Leu), red dotted line] associated with pigmentation is believed to be the mutation under selection.

sweeps where the causal allele is at high frequency (>50%) is even greater, with the causal variant among the top 10 variants in half of cases (table S3).

The CMS results were robust under all demographic scenarios tested (constant population size and bottlenecks of varying strengths), even though the test was optimized for a single model

(6) (fig. S4). The most extreme bottleneck scenarios did increase the number of high-scoring variants in neutral regions, but the false-positive rate remained below 0.01% in all cases (SOM)

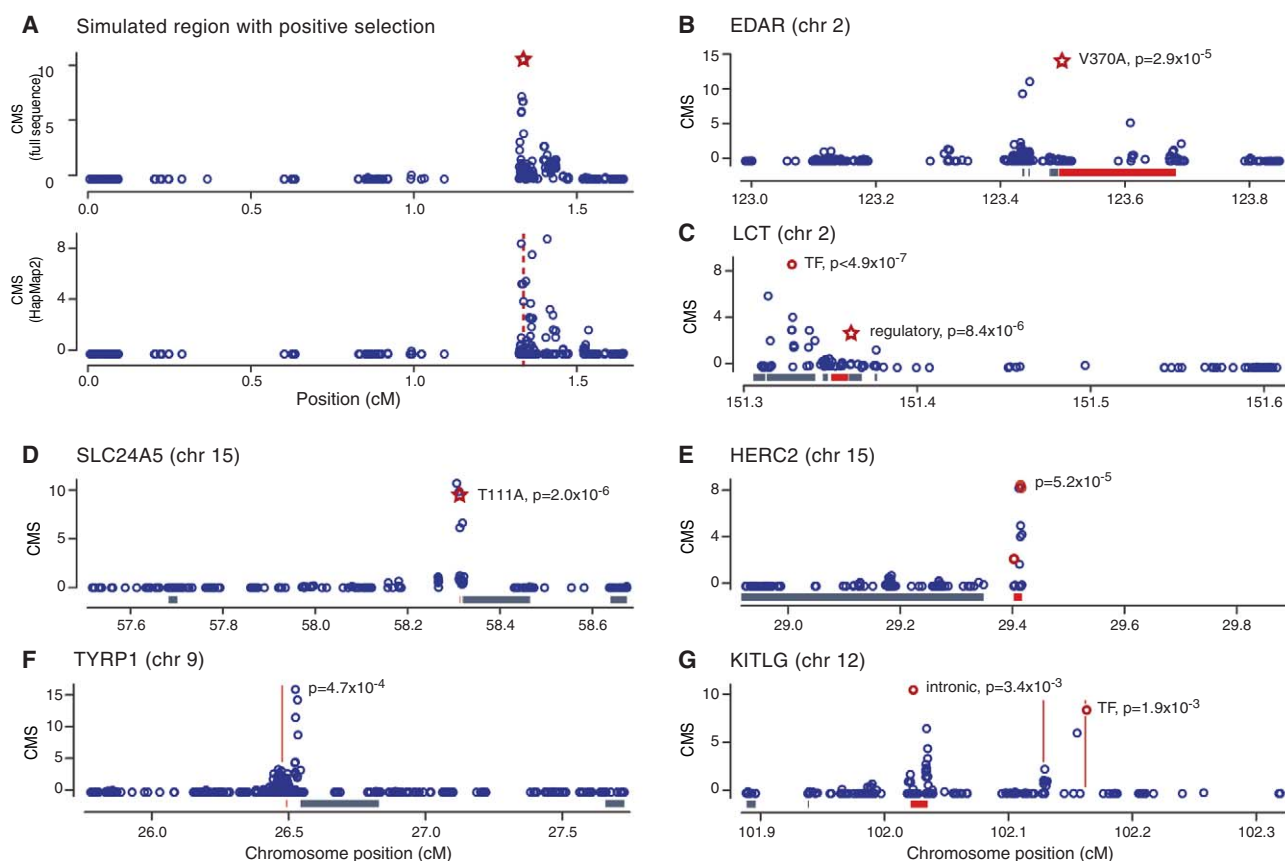
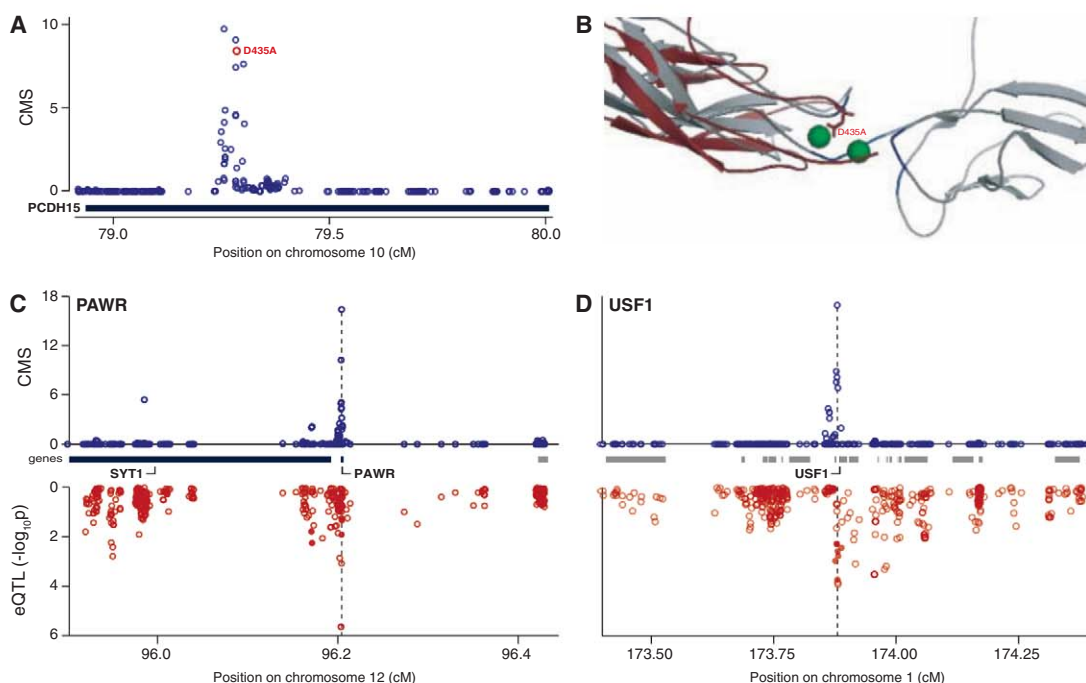


Fig. 3. CMS localizes selection and identifies causal variants in simulated and empirical data. CMS analysis of (A) simulated full sequence and HapMapII-density genotype data sets; and HapMapII selective sweeps at the genes (B) *EDAR*, (C) *LCT*, (D) *SLC24A5*, (E) *OCA2/HERC2*, (F) *TYRP1*, and (G) *KITLG*. Bars

on x axis indicate genes (red bars: putative selected gene; gray bars: other genes); blue circles show CMS values; red stars indicate putative causal alleles; red circles indicate SNPs with annotated function and/or trait association; red lines mark other associated loci.

Fig. 4. Coding and regulatory mutations identified by CMS. (A) CMS scores around *PCDH15* (HapMapII data). Red circle: non-synonymous mutation (D435A). (B) Homology modeling of the *PCDH15* cadherin-4 domain (red) predicts that D435A (red rods) is among the residues (blue) coordinating calcium ions (green) essential to cell-cell adhesion. (C and D) Variants identified by CMS involved in gene regulation. Upper: CMS scores for each HapMapII SNP within the region originally identified as under selection. Lower: strength of association in West African samples between genotype and gene expression level for *PAWR* (C) and *USF1* (D).



(8). These false-positives occurred as isolated points, easily distinguishable from the clearly defined peaks found in selected regions (table S4).

We then applied CMS to empirical human data for 185 candidate regions identified as under recent positive selection in HapMapII data. The data set includes 3.1 million variants genotyped in three populations: Northern Europeans, West Africans (Yoruba from Nigeria), and East Asians (Chinese and Japanese) (7).

As positive controls, we examined several well-characterized regions under positive selection (Figs. 2 and 3). In three regions (containing, respectively, *SLC24A5*, *LCT*, and *EDAR*), a putative causative variant has been previously identified and genotyped in HapMapII (2, 3). In each region, the variant was within the top 10 CMS scores, out of 1000 to 1500 variants in the region. We also examined four regions (350 kb to 1 MB) containing pigmentation-related genes (*MATP*, *TYRP1*, *OCA2* and *HERC2*, and *KITLG*) that are suggested targets of recent selection, but where no candidate variant has been proposed (1, 9, 10). CMS improved the spatial resolution by 3- to 80-fold, and in each case, the narrowed region contains a single pigmentation-related gene. In each case, a strong CMS signal is found at a variant known to be associated in the human population with eye color or skin pigmentation (9).

We then examined the remaining 178 candidate HapMapII regions, containing ~1500 genes, for which the selected locus and variant are unknown. After application of CMS, 64 regions contained a single gene, 35 contained multiple genes, and 79 contained no genes at all. CMS suggested numerous intriguing coding and regulatory functional candidates (figs. S5 and S6 and table S5).

Many regions include striking amino acid changes (table S6). For example, CMS localized a region on chromosome 10 with evidence for selection in East Asians to the protocadherin gene *PCDH15*. The third-highest-ranking variant is an acidic-to-nonpolar (Asp⁴³⁵→Ala) mutation altering a highly conserved residue predicted to lie in the Ca²⁺-binding site at the interface of cadherin repeats in the protein's extracellular domain (SOM) (Fig. 4A and figs. S7 and S8) (11). *PCDH15* plays a role in development of inner-ear hair cells and maintaining retinal photoreceptors (12, 13). Another signal in East Asians localized to the leptin receptor, *LEPR*. The highest-scoring variant is a Lys¹⁰⁹→Arg change in *LEPR* associated with blood pressure, glucose response, and body mass index (14).

Many signals, however, are localized to intergenic regions or regulatory changes in gene regions, suggesting that selected variants may lie in regulatory elements (which also harbor many variants affected in complex diseases). For example, a signal of selection in West Africans localized to a single gene, *PAWR*. Several high-scoring variants show strong association with *PAWR* expression uniquely in West Africans, and with no other genes in the region (fig. S9). Another signal in West Africans localized to a 22-kb region containing two genes, *USF1* and *ARHGAP30*. Several high-scoring single-nucleotide polymorphisms (SNPs) in *USF1* show strong association with *USF1* expression uniquely in West Africans. One variant lies within an experimentally determined transcription factor binding site (15).

Beyond identifying individual gene and polymorphism targets, by reducing the number of genes within each region from about eight to about one, the method reveals instances of multiple genes in the same pathway showing signs of selection. For example, in addition to *PCDH15*, four genes linked to cochlear function or Usher syndrome (1, 16) show evidence for selection in East Asia. We used the PANTHER Gene Ontology database to test for this enrichment on all CMS-localized regions from HapMapII (SOM) (17). We found statistically significant enrichment for several categories (table S7): sensory perception genes (including *PCDH15*) are enriched for selection in East Asia, immune-related genes in West Africa, and genes related to homeostasis and metabolism in all three populations.

CMS can narrow candidate regions for recent local adaptation in humans and identify small numbers of candidate polymorphisms. For this kind of event, we may already be close to the limit on localization from population signals alone. According to our simulations, each causal variant has on average 20 perfect proxies (fig. S10), all essentially indistinguishable from the causal variant. Identifying specific causal variants may thus require functional characterization of small sets of candidates.

The CMS method can be adapted to a wider range of selective regimes, including detecting (i) older selection occurring any time after the divergence of human populations (50,000 to 75,000 years) (F_{ST} and ΔDAF would here become the predominant CMS signals) and (ii) selection on standing variation or very old selection (by incorporating additional population-based tests). It can be applied to nonhuman species with popu-

lation samples of dense genotype or sequence data; as these increasingly become available, the details of the appropriate CMS test would depend on the demographic history and population structure of the species.

Within human genetics, the research community is currently generating data sets of human variation in many populations, through initiatives such as the 1000 Genomes Project (18). With continuing improvements in sequencing technology, it will be possible to examine nearly every variant in the genome in many individuals and populations. With such data emerging for humans and other species, it may be possible to observe much of evolution's most recent handiwork and identify many of the functional adaptations that work to shape species.

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POSITIONS OPEN



FACULTY POSITIONS

Stem Cell and Developmental Biologists

As part of a campuswide expansion in the areas of stem cell biology and developmental genetics at the University of Rochester, the Center for Oral Biology invites applications for two faculty positions at the **ASSISTANT, ASSOCIATE, or FULL PROFESSOR** level.

Qualified applicants in any areas of animal or human developmental biology or genetics are encouraged to apply. Preference will be given to individuals conducting research related to craniofacial development and/or diseases.

Successful candidates at the Assistant Professor level are expected to develop strong, externally funded research programs. Individuals seeking Associate or Full Professor positions should have established outstanding research programs and record of extramural funding.

The Center of Oral Biology is located in the state-of-the-art Arthur Kornberg Medical Research Building at the University of Rochester School of Medicine and Dentistry.

Faculty members in the Center carry joint appointments in appropriate academic departments and participate in graduate student training in several graduate programs in the University of Rochester.

More information about the Center for Oral Biology and the University of Rochester School of Medicine and Dentistry can be found on the Internet ([website: http://www.urmc.rochester.edu](http://www.urmc.rochester.edu)).

Applications will be accepted immediately and until positions are filled. Please send curriculum vitae, statement of current and future research interests, and names and addresses of at least three references to: **Dr. Robert Quivey, Director, Center for Oral Biology, Box 611, University of Rochester Medical Center, 601 Elmwood Avenue, Rochester, NY 14642.** *The University of Rochester is an Equal Opportunity Employer. Women and minorities are encouraged to apply.*

ASSISTANT PROFESSOR

Department of Physiology and Biophysics Dalhousie University

The Department of Physiology and Biophysics invites applications for a position at the rank of Assistant Professor for an individual with research interests in the electrophysiology of excitable cells and tissues. Candidates whose research incorporates modern molecular biology and imaging techniques will be given preference. Applicants must have a Ph.D., M.D., or equivalent degree, postdoctoral training, excellent communication skills, and a strong record of peer-reviewed publications. The successful candidate will be expected to develop active and synergistic research collaborations with other basic science or clinical researchers in the Faculty of Medicine, to develop an extramurally funded research program, and to participate in the teaching mission of the Department. This is a salaried, probationary, tenure-track appointment; however, the candidate will be encouraged to apply for external salary support from appropriate granting agencies.

Interested applicants should submit curriculum vitae along with a brief description of research and teaching experience and interests, and should arrange to have three letters of reference sent directly to: **Dr. Paul R. Murphy, Head, Department of Physiology and Biophysics, Faculty of Medicine, Dalhousie University, Sir Charles Tupper Medical Building, 5850 College Street, Halifax, Nova Scotia B3H 1X5, Canada.**

Applications must be received by February 28, 2010, to receive full consideration.

All qualified candidates are encouraged to apply; however, Canadians and permanent residents will be given priority. *Dalhousie University is an Employment Equity/Affirmative Action Employer. The University encourages applications from qualified Aboriginal people, persons with a disability, racially visible persons, and women.*

POSITIONS OPEN



FACULTY POSITION

Department of Biochemistry

Stanford University School of Medicine

Applications or nominations are invited for an **ASSISTANT PROFESSOR** position in the Department of Biochemistry. Applicants should have an established record of excellence in original research. The principal criterion for appointment in the University Tenure Line is a major commitment to research and teaching. Candidates should submit curriculum vitae including a list of publications, a description of their research interests, and names, addresses, telephone numbers, and e-mail addresses of three references to **e-mail: biochemistry_recruitment@stanford.edu**. Applications should be received by March 15, 2010. Reference letters should be sent to the above e-mail address or to: **Search Committee Chair, Department of Biochemistry, Stanford University School of Medicine, 279 Campus Drive Room B400, Stanford, CA 94305-5307.**

Stanford University is an Equal Opportunity Employer and is committed to increasing the diversity of its faculty. It welcomes nominations of and applications from women and members of minority groups, as well as others who would bring additional dimensions to the University's research, teaching, and clinical missions.

SENIOR FACULTY POSITION Pain Research

The Department of Anesthesiology and Perioperative Care at the University of California, Irvine is seeking laboratory-based Ph.D., M.D., or M.D.-Ph.D faculty members with an interest in pain research. A tenure opening is available at the **ASSOCIATE** or full **PROFESSOR** level. Candidates are expected to have an established and independently funded laboratory research program using molecular, cellular, genetic, electrophysiological, and/or imaging approaches to advance the understanding of the neurobiology and treatment of pain. We will consider applicants in any area of neuroscience, including neurodegeneration and repair, neuroanatomy, and stem cell biology; however, a research focus on pain is necessary. Priority will be given to applicants with research programs of direct clinical relevance, and to physician-scientists with clinical expertise in addition to scientific accomplishments.

We invite applicants who currently hold a position at the rank of Associate Professor or Professor. Successful candidates must have a Ph.D. and/or M.D. or equivalent degree and an established record of independent research, scholarly activity/publications, including existing extramural funding. Investigators with active R01 awards or equivalent funding are encouraged to apply. Those selected will be expected to further develop, manage, and maintain their own independent, extramurally funded research program(s). The successful candidates must have the capacity to thrive in a multidisciplinary research and teaching environment.

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REACHING GENDER EQUITY IN SCIENCE:

THE IMPORTANCE OF ROLE MODELS AND MENTORS

The number of women embarking on science careers has been increasing steadily during the past several decades. Although women scientists continue to be underrepresented at the faculty level, many women have established rewarding and successful careers in science—thanks in part to having had role models and mentors whose paths they could follow. **By Laura Bonetta**

Judith Weis knows about the value of role models. Last spring, the biology professor received a heartwarming e-mail from a woman who had been inspired to pursue a career in science by a television commercial—one that Weis had starred in.

In the 1970s the makers of a popular orange beverage called Tang shot a series of television commercials featuring women scientists with their children. Weis, who had recently joined the faculty at Rutgers University in New Jersey, took part in one of them. The e-mail writer saw the commercial as a child and went on to obtain a Ph.D. in pathology in 1987. “I vividly remember the commercials and thinking—I can do that!” she wrote. “So, thank you—for deciding to make the commercial and become a positive role model, not only for me but hopefully many other women.”

When Weis started her research career in marine biology, there were few women scientists, particularly at the faculty level. “The department I joined was exceptional because it had three tenure-track female faculty,” she says. “It was very unusual for back then.” The situation has changed dramatically in the past three decades. “Up until the 1970s women were not even allowed to go out on oceanographic vessels. In the 1980s things changed a lot. I remember one of the first signs was going to a conference and there was a line for the women’s bathroom,” recalls Weis laughing. “Since then women have been flocking to marine biology.”

Weis’s experience parallels national trends in the United States. According to the latest figures from the NSF (National Science Foundation)—Women, Minorities and Persons with Disabilities in Science and Engineering, 2009—in 2006 women accounted for more than half of all graduate students in some science fields—76 percent of graduate students in psychology, 56 percent in biological sciences, and 54 percent in social sciences. (But women made up only 23 percent of graduate students in engineering and 25 percent in computer science.) Women also accounted for a rising share of postdocs in all fields except computer sciences; in 2006, 53 percent of psychology postdocs, 46 percent of social sciences postdocs, and 41 percent of biological sciences postdocs were women.

At the faculty level, however, change has been slower. According to the report released in June 2009 by the National Research Council, National Academy of Sciences (NRC), Gender Differences at Critical Transitions in the Careers of Science, Engineering and



Marcie McClure

“There is a movement toward more gender equity than noted in previous reports or often publicly appreciated. At the same time, the findings show that we are not there yet.”

Mathematics Faculty, women are not applying for tenure-track jobs at research-intensive universities at the same rate that they are earning Ph.D.s. For example, while women received 45 percent of the Ph.D.s in biology from 1999 to 2003, they accounted for 26 percent of applicants to tenure-track positions.

European figures are similar. Preliminary findings of the She Figures 2009: Women in Science Across Europe report, produced by the European Commission, reveal that in 2006, 45 percent of students graduating in the European Union at Ph.D./Doctorate level were women—an improvement over the 2002 figure of 42 percent. (There were, however, significant differences between disciplines, with women being noticeably less represented in the physical sciences and engineering, compared to life and social sciences.)

But as in the United States, career retention is an issue for European female researchers. The EU average of women in Grade A research positions—the single highest post at which research is conducted—in 2007 was only 20 percent. This is an improvement from 2002, when this figure was 17 percent.

Having Role Models

One of the factors that has inspired more women to pursue scientific careers has been having examples of successful women who have done the same. “When you are 24 or 26 and are looking at different career options—industry, academia, or government labs—men see three clear paths and will know several people who **continued »**

UPCOMING FEATURES

Postdoc 1: Life Beyond the Bench—March 5

Faculty 1: Lab Management—March 12

Careers in Bioinformatics/Systems Biology—April 9

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The successful candidate will be appointed research group leader in the Department of Biomedicine (<http://biomedizin.unibas.ch/>) and is expected to be a major driving force in the stem cells and regenerative medicine research focus. The successful applicant should have several years of postdoctoral research experience at the forefront of stem cell research, tissue engineering and/or regenerative medicine, and should belong to the promising young talents with an already excellent publication record. Expertise in the generation/isolation and directed differentiation of induced pluripotent stem cells (iPS cells) or adult stem cells from humans or mice would be desirable.

The main task of the newly appointed Professor will be to establish a competitive research program with international visibility and teach stem cell biology. The Network of Excellence in Life Sciences (<http://lifesciences.unibas.ch/>) will provide the new stem cell research group with an outstanding interfaculty research environment. The teaching language is German and successful candidates are expected to learn German within 2–3 years.

The University of Basel seeks to increase the proportion of women among the faculty members and therefore specifically encourages female candidates to apply. Information about how to submit your application is available at:

<http://medizin.unibas.ch/dekanat/bewerbungen.html>.

Complete applications must be sent as pdf-files on a compact disc to: Medizinisches Dekanat der Universität Basel, Klingelbergstrasse 61, CH-4056 Basel, Switzerland

Application deadline is 9.4.2010

For further information, please contact: Prof. Rolf Zeller (e-mail: rolf.zeller@unibas.ch).



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www.helmholtz.de/yig

DEADLINES:

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The Helmholtz Association is an equal opportunity employer and is committed to increasing the percentage of women in group leader positions.

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DIVERSITY: WOMEN IN SCIENCE



"For those of us with many years of experience in this scientific endeavor, it is critically important that we serve as mentors."

— Geraldine Richmond

traversed each one. They can see other men 20 years down the line," says **Geraldine Richmond**, a professor of chemistry at the University of Oregon. "But for women it is more of a fog. They may not know anyone who has gone that road, or at least no other women. So they cannot visualize where they are going to go. If you plan to have children, but don't see any women who have gone that path, you may not be sure it's possible."

To help provide role models for young women scientists, Richmond co-founded the Committee on the Advancement of Women in Chemistry (COACH)—a program that provides dozens of professional development workshops and networking events each year for women scientists and engineers around the country. So far the COACH workshops have impacted the careers of over 4,000 women. "In addition to the training, women have the opportunity to share their challenges and successes, helping to make those paths less foggy," says Richmond.

Richmond is herself a mentor and role model to many students and postdocs in her department and around the country. "For those of us with many years of experience in this scientific endeavor, it is critically important that we serve as mentors to others that are following in our footsteps, helping them to identify the career path that best fits their values and aspirations, and to help them to succeed," she says.

Having role models may be particularly critical in fields where there are fewer women. **Aoife Moloney**, a lecturer at the School of Electronic and Communications Engineering at Dublin Institute of Technology in Ireland, has always been in the minority. "It's something you have to get used to," she says. "I have been used to it for the most part of my life. Few women do math and physics."

To help get more women into the field, she takes part in Role Model Day at her institute. "That has been very successful. We invite women engineers working in industry mainly. They talk to second level students about doing engineering," explains Moloney. "We always inspire a few students." And although being the sole woman in a department can be off-putting to some, Moloney does not think that prospect should keep women away from engineering. "The field is welcoming to women," she says. "Men like to have female colleagues. They enjoy working in a more mixed environment."

Marcie McClure, a professor in the Department of Microbiology at Montana State University, Bozeman, came up with a different strategy to introduce young students to role models in her field. "Four years ago I was attending a conference in Brazil. I was in the swimming pool with some students who were saying that there was no place for women in bioinformatics. They thought the field was too

cutthroat and male dominated," recalls McClure. She realized that those students did not know that many of the pioneers in the field of bioinformatics had been women.

As a result, she decided to make a documentary of interviews with prominent women scientists. "We filmed these women as we asked them about their youth, their being mentored, why they became fascinated in science," says McClure. "I wanted young women to know that there is a place for them in science."

The videos are now available online through SciVee TV (<http://www.scivee.tv/user/womeninbioinformatics>).

The Importance of Mentors

The NRC's committee in charge of the Gender Differences report determined that women who had a mentor did better than women without one.

They reached this conclusion by analyzing the results of two national surveys, taken in 2004 and 2005, of tenure-track and tenured faculty in six disciplines (biology, chemistry, mathematics, civil engineering, electrical engineering, and physics) at 89 institutions. They found that in chemistry, for example, female assistant professors with mentors had a 95 percent probability of having grant funding versus 77 percent for those women without mentors. Across the six fields surveyed, female assistant professors with no mentors had 68 percent probability of having grant funding versus 93 percent of women with mentors.

Stem cell researcher **Amy Wagers** realized early in her career the importance of "having the perspective of people you can trust." Part of the reason she accepted a faculty position at Harvard Medical School was that she thought she would have good mentors there. "Even as I was going through the interviewing process I was thinking, Who would be a good mentor?" she says. "I was looking for people who seemed genuinely interested in my work and also in mentorship." She now has several mentors in her department, as well as outside her institution.

Wagers herself has since become a mentor to numerous graduate students and postdocs, many of whom are looking for examples of successful scientists who have achieved a healthy work-life balance. "Some graduate students will ask me, Is it more of a challenge for a woman to do science?" she says. "But it's the same for everyone. You really have to love what you are doing. Then it's not a challenge but an opportunity."

To increase the chances that scientists will benefit from mentoring, a number of universities and national organizations have created programs to formalize the process. For example, the Association of Women in Science has many chapters around the country that bring women scientists together to network. For scientists who cannot meet in person, MentorNet is an online service that virtually connects established scientists with undergraduates and graduate students, postdocs, and beginning faculty (www.mentornet.com). In Europe the Max Planck Institute of Biophysics in Frankfurt, Germany, set up "Minerva-FemmeNet," a network for female scientists at www.mpibp-frankfurt.mpg.de/misc.

The NSF provides grants through ADVANCE (which stands for Increasing the Participation and Advancement **continued** »



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Michigan State University is committed to achieving excellence through cultural diversity. The university actively encourages applications and/or nominations of women, persons of color, veterans and persons with disabilities.

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www.mpg.de/english

Montana State University
www.montana.edu

National Research Council
sites.nationalacademies.org/NRC

National Science Foundation
www.nsf.gov

University of Michigan
www.umich.edu

University of Mississippi
www.olemiss.edu

University of Oregon
www.uoregon.edu

Yale University School of Medicine
medicine.yale.edu

"We are trying to get women scientists to network more and encourage them to seek multiple mentors."

— Laura Olsen



Reckelhoff, who received a Ph.D. in biochemistry from the Medical College of Virginia at the Virginia Commonwealth University in 1985, says she was fortunate that her graduate adviser was a very good mentor and taught her what proper mentoring is about. "She taught me everything, from writing papers to how to conduct myself at a meeting," recalls Reckelhoff. "She was very involved in what I was doing."

Since then Reckelhoff has had many mentors, both men and women, and she is herself a mentor to several postdocs. "They come to me about anything having to do with research, to interactions with people in the departments, to interpersonal relationships. I have the same types of conversations with female and male graduate students," she says.

Moving Forward

The data show that much progress has been made in advancing the careers of women scientists. The proportion of female graduate students and postdocs in most scientific fields is higher than it's ever been. And although women scientists are in the minority at the faculty level, women faculty tend to be as successful as their male colleagues. According to findings reported in the NRC's Gender Differences, although women are underrepresented in the applicant pool for faculty positions in many fields, those who do apply are hired at rates equal to or higher than those for men. Similarly, although fewer women are considered for tenure compared to men, those who are receive the promotion at rates equal to or higher than men.

Despite such progress, there are still challenges. As **Sally Shaywitz** of the Yale Center for Dyslexia and Creativity at Yale University School of Medicine said in a press release accompanying the NRC report, "There is a movement toward more gender equity than noted in previous reports or often publicly appreciated. At the same time, the findings show that we are not there yet. The gap between female graduates and the pool of female applicants is very real, and suggests that focus next be placed on examining challenges such as family and child responsibilities, which typically impact women more than men."

While it may take some time to reach true gender equity in science—as well as other professional fields—role models and mentors will continue to play important roles for moving in the right direction.

Laura Bonetta is a scientist turned freelance writer based in the Washington, D.C., area.

DOI: 10.1126/science.opms.r1000084

of Women in Academic Science and Engineering Careers)—a program that supports the development of systemic approaches to increasing the representation and advancement of women in academic science, technology, engineering, and mathematics. Many such approaches, funded through ADVANCE, include a mentoring or advising program.

The University of Michigan received an ADVANCE grant for its Supporting Women Scientists and Engineers program, which includes career advising, networking opportunities, discussions, as well as grant opportunities. "We are trying to get women scientists to network more and encourage them to seek multiple mentors," says **Laura Olsen**, associate chair of Molecular, Cellular, and Developmental Biology and one of the program's career advisers.

Given the growing awareness of the importance of mentoring and the number of programs to support mentoring programs, it is perhaps surprising that many faculty members lack mentors. The NRC's surveys asked tenure-track faculty and faculty tenured after 2001 whether they had or have a faculty mentor at their current institution. Among tenure-track faculty, 49 percent of the men and 57 percent of the women reported having a faculty mentor. Among recently tenured faculty 45 percent of men and 51 percent of women reported having a faculty mentor.

And although women were more likely than men to have mentors, they reported being less likely than men to engage in conversations with their colleagues on a wide range of professional topics including research, salary, and benefits. "This distance may prevent women from accessing important information and may make them feel less included and more marginalized in their lives," says the report.

"Regardless of whether you are a man or a woman, you will need mentors at every level of your career," says **Jane Reckelhoff**, a professor in the Department of Physiology at the University of Mississippi Medical Center in Jackson. "Even the chair of the department needs mentors."



ENDOWED PROFESSORSHIP DEPARTMENT OF BIOMEDICAL ENGINEERING

The Department of Biomedical Engineering in the College of Engineering at the University of Michigan is searching for an outstanding senior faculty candidate for an endowed professorship in cancer research. The successful candidate will join a vibrant bioengineering community with strengths in biomaterials, biosensors, tissue engineering, biofluid mechanics, microfluidics, biomolecular engineering, molecular and energy-based therapeutics, biomedical imaging and optics, neural engineering, bioMEMS, and micro/nano biotechnology. We expect the successful candidate will interface closely with an outstanding research and clinical cancer community at the University of Michigan Medical School. Qualifications include an earned doctorate in a related discipline and demonstrated excellence and leadership in, and commitment to, teaching, research, and scholarship.

Ann Arbor is among the most attractive cities in the country, offering many cultural, artistic, musical, recreational and educational activities. The University of Michigan is a premier public university with top-rated Engineering, Medical, Law and Business programs, and is responsive to the needs of dual career families. The College of Engineering is dedicated to the goal of building a culturally diverse and pluralistic faculty committed to teaching and working in a multicultural environment.

Applicants should send a letter of interest with curriculum vitae and a list of references to:

Biomedical Engineering Search Committee
Biomedical Engineering
The University of Michigan
1107 Carl A. Gerstacker Building
2200 Bonisteel Blvd.
Ann Arbor, MI 48109-2099
e-mail: bmerecruit@umich.edu

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To be held in any School within the Faculty of Sciences, this Fellowship is available for a 4 year period to Australian citizens with a doctorate or equivalent research experience. Fellows will be expected and encouraged to establish their own independent research program and funding. Start up funds will be provided.

Closing date 9/04/2010

More information:

For research within the faculty:
www.sciences.adelaide.edu.au/research

For further information and to apply please contact Professor Bob Hill, Executive Dean, Faculty of Sciences, telephone: +61 8 8303 5650, or email: execdean.facsociences@adelaide.edu.au

Applicants must address the selection criteria for the position, available from

www.adelaide.edu.au/jobs

Young Australian Scientists working overseas are particularly encouraged to apply.



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DIVERSITY

The Berlin Aging Study (BASE) II seeks

postdoctoral fellow (for two years, starting immediately)

with expertise in biostatistics/genetic epidemiology. Main tasks: Performing statistical analyses of genomewide association (GWAS) data in relation to behavioral and brain data. Requirements: Ph.D. degree and/or postdoctoral experience in genetic epidemiology, genetics, statistical genetics, or medicine; experience in analyzing GWAS data (e.g., with PLINK, PBAT), and experience in extensive data integration, management, and database mining (e.g., using R or Perl). Experience in imputation of untyped genotypes from GWAS data and analysis of next-generation sequencing data is desirable.

The project is a collaboration between the Max Planck Institute for Human Development <www.mpib-berlin.mpg.de> and the MPI for Molecular Genetics <www.molgen.mpg.de>, and several local research institutions.

The Max Planck Society is interested in increasing the number of women on its scientific staff and strongly encourages applications from women and members of minority groups. It is also committed to employing more handicapped individuals and particularly encourages them to apply.

Please email your application (statement of research interests, CV, incl. publication list, and two references) to Dr. Lars Bertram (lbertram@molgen.mpg.de) and Prof. Dr. Shu-Chen Li (shuchen@mpib-berlin.mpg.de). Applications will be processed until the position is filled.



MAX-PLANCK-GESELLSCHAFT

TRUDEAU INSTITUTE

IMPROVING HEALTH THROUGH MEDICAL RESEARCH

FACULTY APPOINTMENTS IN IMMUNOLOGY AND INFECTIOUS DISEASE

The Trudeau Institute is recruiting new faculty at junior and senior levels with interest in the areas of immunology and infectious disease. We are looking for individuals who will develop vigorous, extramurally funded, research programs that will integrate with the highly collaborative scientific environment at the Institute (www.trudeauinstitute.org). Applications from related disciplines are encouraged.

The Institute offers excellent, well-equipped, laboratory space and extensive shared facilities, including animal, molecular biology, flow cytometry, bio-imaging, and histology cores. A modern, state-of-the-art animal facility offers skilled colony maintenance with database tracking, on-site genetic screening and a wide variety of standard and genetically altered mice at subsidized rates. The Institute has excellent containment facilities for in vitro and in vivo BSL2 and BSL3 activities and is poised to open a brand new research facility that is select agent capable. The Institute is partially supported by an endowment and offers competitive start-up packages with extended support, on-campus housing, an on-site child-care center and an excellent benefits package.

Interested individuals should submit a letter of application, including a description of research interests, curriculum vitae, and contact information for three references to the address below. Application deadline is **April 30, 2010**.

Applications should be addressed to: **Dr. Andrea Cooper, Chair, Faculty Search Committee, Trudeau Institute, Saranac Lake, NY 12983**. E-mail applications are preferred. Please send to faculty_chair@trudeauinstitute.org.

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Equal Opportunity/Affirmative Action Employer.*



**Department of Health and Human Services
National Institutes of Health
Office of the Director, NIH**



Deputy Director for Extramural Research, NIH

The National Institutes of Health (NIH) in Bethesda, Maryland, the world's premier biomedical research institution, is seeking applications from exceptional candidates for the exciting position of Deputy Director for Extramural Research (DDER). The DDER is the principal scientific leader and advisor to the Director, NIH, on all matters relating to the substance, quality, and effectiveness of the NIH extramural research program and administration. As one of four Deputy Directors of the agency, the DDER is a member of NIH's senior executive leadership and works closely with the Director and the Principal Deputy Director in providing significant input on major issues affecting the NIH, and developing strategic policies and implementation plans for advancing the NIH mission. As the leader for NIH's extramural research, the DDER is responsible for all matters involving NIH research grant policy and administration, and as such, serves as a key spokesperson for NIH to the Congress, the press, and scientific organizations. The DDER has recently played a key role in: developing and implementing the NIH Public Access policy, orchestrating the implementation of the American Recovery and Reinvestment Act, enhancing oversight of existing financial conflict of interest policies and regulations, increasing the transparency of the NIH research portfolio through development of user-friendly web tools, and developing the policies and tools to support the electronic submission of grant applications.

The position also serves as the Director of the Office of Extramural Research (OER) providing supervision of and leadership to approximately 1,200 OER employees and contract staff, ensuring appropriate day-to-day operations and commitment to work force diversity enhancement. This office has lead responsibility for the development and implementation of NIH peer review, programmatic, and grants policies, overseeing the development and implementation of policies on the humane use and care of laboratory animals, coordination with the Department of Health and Human Services on human subjects research protection, and development and maintenance of the information systems for NIH grants administration. The OER has an estimated budget of more than \$110 million for FY2010.

Salary is commensurate with experience, and a full package of Civil Service benefits is available including retirement, health and life insurance, long-term care insurance, leave, and savings plan (401k equivalent). A detailed vacancy announcement that includes mandatory qualifications requirements and application procedures can be accessed at NIH's Executive Jobs Site: <http://www.jobs.nih.gov/vacancies/executive.htm> or by contacting Patti Brown at (301) 402-9459. CV, bibliography, and a letter of interest, including a section addressing the qualifications requirements must be received by close of business **March 10, 2010**.

DHHS and NIH are Equal Opportunity Employers

Full Professor Positions

As part of its expanding commitment to biomedical research, bioengineering, and translational science, Northeastern University in Boston, MA is seeking two outstanding senior hires for joint faculty positions at the rank of Full Professor. Northeastern University is located four blocks from the Longwood Medical and Academic Area, encompassing Harvard Medical School, Children's Hospital, Brigham & Women's Hospital, Dana-Farber Cancer Institute, and Beth Israel Deaconess Medical Center, and eight blocks from the Boston University School of Medicine and Boston Medical Center.

Drug Discovery Position

The Drug Discovery position will be jointly held in the Bouvé College of Health Sciences and the College of Sciences, with appointment in the Center for Drug Discovery. The ideal candidate will be a recognized educator and leading biomedical researcher with an active research program in understanding disease mechanisms and discovering novel pharmaco-therapeutic agents.

More information regarding this position may be obtained by contacting the Search Committee Chair, Professor Roger Giese at r.giese@neu.edu.

Tissue Engineering/Regenerative Medicine Position

The Tissue Engineering/Regenerative Medicine position will be jointly held in the Bouvé College of Health Sciences and the College of Engineering. The ideal candidate will be a recognized leader in the biosciences and bioengineering, specifically in the area of stem cells, tissue engineering, and regenerative medicine.

More information regarding this position may be obtained by contacting the Search Committee Chair, Professor Craig Ferris at c.ferris@neu.edu.

Salary is commensurate with education, training, and experience, and includes an outstanding benefits package. The start date is September, 2010. Evaluation of candidates is ongoing and applications are accepted until the position is filled.

How to Apply: Applications must be submitted online by visiting the Provost and College website at <http://www.northeastern.edu/provost/faculty/positions.html> and clicking on 'Access Faculty Positions'. Applications and nominations will include a cover letter, a statement of current and future research interests, curriculum vitae, and contact information for at least four references.

Northeastern University Equal Employment Opportunity Policy: Northeastern University is an Equal Opportunity/Affirmative Action, Title IX, and an ADVANCE institution. Minorities, women, and persons with disabilities are strongly encouraged to apply. Northeastern University embraces the wealth of diversity represented in our community and seeks to enhance it at all levels.



<http://www.neu.edu>



PHARMACOLOGY/TOXICOLOGY FACULTY POSITION University of Missouri-Columbia Department of Medical Pharmacology and Physiology

The Department of Medical Pharmacology and Physiology, University of Missouri-Columbia invites applications for a tenured or tenure-track position (**Assistant to Full Professor**). The successful candidate must have a PhD in Pharmacology, Toxicology, or a related field or an MD, postdoctoral experience, and significant extramural grant support. Preference will be given to candidates with expertise in molecular pharmacology or toxicology who are interested in working in a highly collaborative, interdisciplinary environment and whose research interests will complement and expand existing areas of departmental research strength in cardiovascular function, metabolic syndrome/diabetes, cellular signaling, and receptor or channel activity. Successful applicants will be expected to maintain an active research program that is supported by significant extramural funding and contribute to departmental teaching activities. Involvement in campus-wide research initiatives relative to cardiovascular health and diabetes, gender physiology and environmental adaptation, or nutrition and exercise science is desirable. The position is supported by an excellent start-up package, competitive compensation and benefits, newly renovated laboratory space, and access to state-of-the-art core facilities. There are excellent opportunities for collaboration at the molecular, cellular, and systems levels of integration. The University is noted for its robust interdisciplinary research programs and is located in Columbia, a delightful, vibrant, family-oriented "college town" that is consistently named to several "Best Small Cities" lists, providing affordable housing, quality K-12 education, and an active cultural community, all located within the scenic rolling hills of central Missouri.

Please send a curriculum vitae that includes previous and current research funding, a narrative description of research and educational interests, and the names and contact information of three references by electronic submission (strongly preferred) to: MPPsearch@health.missouri.edu or by mail to: **Chair - MPP Search Committee, Department of Medical Pharmacology and Physiology, MA415 Medical Sciences, University of Missouri-Columbia School of Medicine, One Hospital Drive, Columbia, MO 65212.** Active review of applications will begin immediately and the search will continue until the position is filled.

The University of Missouri-Columbia is an EEO/AA/ADA Employer. Please direct ADA accommodation requests to our coordinator at (573) 884-7278 (V/TTY).



Director, Participant and Clinical Interactions Resource (Clinical Research Center) Meharry Center for Translational and Clinical Research (MeTRC) Meharry Medical College, Nashville, Tennessee

As Director of the Meharry Participant and Clinical Interactions Resource (PCIR), (Clinical Research Center), the candidate will provide research and clinical trial expertise for ongoing and future clinical studies to investigators, sponsors, research professionals and funding agencies. The PCIR Director is also responsible for the daily management and operations of clinical research including financial, regulatory and compliance services for all Meharry clinical research activities. S/he will also be available to serve as collaborator or co-investigator on NIH-funded investigator-initiated and pharmaceutical clinical studies and provide medical and research expertise for studies conducted at PCIR. The Director will oversee and assume responsibility for clinical trial billing compliance to trial sponsors, appropriate identification of charges billable to third party payors, contract negotiation, budget development, feasibility analysis, regulatory support and ongoing post-award management for clinical trials. The PCIR Director will also serve as a clinician educator having responsibility for both teaching and patient care in the relevant medical or surgical specialty. S/He will be expected to make a strong commitment to training students, residents, fellows and junior faculty. This commitment will include helping to coordinate and facilitate development of their careers within the field of clinical and translational research. The Director will also ensure that the PCIR provides knowledgeable, professional and timely support to faculty and staff. The PCIR Director will also be responsible for safeguarding Meharry's interests by assuring compliance with various Federal and State regulations and laws, as well as Institutional policies associated with management of clinical research. S/He will be responsible for developing PCIR policies and procedures relating to corporate sponsored clinical research. The Director will be responsible for developing, organizing, participating in, and/or conducting portions of orientations, workshops, presentations, etc. regarding sponsored clinical research, proposal writing and submissions. Meharry has built a strong foundation of research in the areas of infection, immunity and women's health. Therefore we are especially interested in clinical scientists who study infectious diseases and women health including HIV/AIDS. Requirements include an MD degree and board certification in an appropriate medical or surgical specialty with a minimum of 5 years of clinical research, and current or recent (within last 2 years) independent NIH research funding. A strong clinical research regulatory background is essential. Knowledge of clinical research regulations and Federal and State law governing clinical research and tax-exempt organizations is required. Knowledge of Federal healthcare and commercial insurer billing and coding manuals and regulations is strongly desired. Knowledge of computer databases and general computing software is essential, and experience with clinical trial management systems is desired. Academic appointment and rank will be commensurate with experience, accomplishments, and expertise. Applicants are encouraged to submit their resumes to **Dr. James E.K. Hildreth, Director, Center for AIDS Health Disparities Research** via email to: jhildreth@mmc.edu.

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Starting this year, KTH will expand research activities in six strategic areas, thereby deepening our impact on the world's great challenges. We are looking for international calibre research talent to join us.

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- Information and communication technology
- e-Science
- Molecular biosciences
- Transportation
- Production engineering
- Energy

Please visit www.kth.se/sra for more information about the available positions, our research and KTH

KTH, founded in 1827, is Sweden's leading technical university. We account for one-third of Sweden's technical research and engineering education capacity at a university level. Education and research cover a broad spectrum – from the natural sciences through all the branches of engineering, as well as architecture, industrial engineering and management, urban planning, work science and environmental engineering.



ROYAL INSTITUTE
OF TECHNOLOGY

PHOTO: KTH/ERICSSON LINDBLAD



FOOD BIO-PROCESSING UNIT, MOHALI, PUNJAB

An autonomous Institution being set up by Deptt. of Biotechnology
Ministry of Science & Technology, Govt. of India

RECRUITMENT FOR THE POSITION OF CHIEF EXECUTIVE OFFICER - 1 POST

The Department of Biotechnology, Ministry of Science & Technology, Government of India is establishing an Agri-food cluster comprising of NATIONAL AGRI-FOOD BIOTECHNOLOGY INSTITUTE and FOOD BIOPROCESSING UNIT (FBPU) along with an Agri-food Biotechnology Park in the "Knowledge City", Sector 81, Mohali, Punjab. The FBPU will be a real time interface facility between the laboratory scale testing and large scale manufacture. The FBPU shall link the R&D system with a production facility to serve as an incubator for start-ups. BPU will produce pilot lots under GMP of new product for clinical testing for safety and validation of claimed nutritional/health benefits.

FBPU is looking for an exceptional candidate for the post of **Chief Executive Officer (CEO)** to spearhead the establishment of the programme. The position offers a unique opportunity for the right individual in providing strong and visionary leadership to the programme.

Professional Requirements: The candidate must have a Ph.D. or equivalent degree in Food Technology/ Biochemical or Bioprocess Engineering or related field; knowledge of product and process development and related technologies along with having working experience in a food processing unit. He/She must have at least **15 years'** research experience, be able to interact nationally and internationally.

Salary & Allowances: Basic Rs. 80,000/- (revised). Allowances and other perks will be as per approved norms of the Government of India.

As a founder CEO, the responsibilities would include: 1) leading and nurturing the core multi-disciplinary programme 2) developing policies and fostering collaboration with scientific national/ international partner institutions and agencies 3) developing mechanism for collaboration with food processing industry 4) promotion of entrepreneurship in the area 5) recruitment of appropriate faculty

How to Apply: The applications duly forwarded by the respective institutions, along with details, i) Curriculum Vitae indicating the date of birth, ii) address for correspondence including telephone, fax and e-mail address, iii) qualifications acquired, iv) professional and research experience, v) present position and scale of pay with total emoluments, vi) publication details along with H index and vii) a write-up on the candidate's vision of how FBPU should be developed and the vision for the next ten years, super-scribing the cover "**Application for the Post of Chief Executive Officer (FBPU)**", be sent to:

Dr. Rajesh Kapur, Advisor (Food & Nutrition)
Department of Biotechnology
Ministry of Science & Technology, Block-2,
CGO Complex, Lodi Road, New Delhi – 110 003

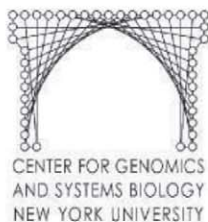
Last Date for Submission of Application
31st March 2010



NEW YORK UNIVERSITY

CLINICAL ASSISTANT PROFESSOR & RESEARCH AFFAIR COORDINATOR

Center for Genomics & Systems Biology
DEPARTMENT OF BIOLOGY



The Center for Genomics and Systems Biology (CGSB) within the Department of Biology at New York University invites applications for a Clinical Assistant Professor & Research Affairs Coordinator appointment to start September 1, 2010, pending budgetary and administrative approval. In close collaboration with the CGSB Director, the successful candidate will develop and communicate a strategic vision for the Center, raise funds, and provide outreach to faculty within the Faculty of Arts and Sciences and the University. The candidate will also develop and teach 4 courses per year in areas such as genomics, systems biology, science ethics, or grant writing.

The successful candidate will have a broad scientific background, with a Ph.D. in biology or related fields. Familiarity with the field of genomics and systems biology is essential. Other requirements are excellent written and verbal communication skills; experience in grant writing; the ability to communicate with scientific and lay audiences; strong general administrative skills; and a demonstrated ability to manage a program of this scope. Experience in teaching is preferred.

Candidates should submit a single PDF file containing a Cover Letter and CV to **biology.research@nyu.edu**. The following address can be used for the cover letter: **Dr. Fabio Piano, Center for Genomics and Systems Biology, New York University, 1009 Silver Center, 100 Washington Square East, New York, NY 10003**. *Three PDF letters of reference should also be submitted separately to **biology.research@nyu.edu**. Closing date for all materials is **March 12, 2010**.*

NYU is an Equal Opportunity/Affirmative Action Employer.



European Chemicals Agency

The **European Chemicals Agency (ECHA)** is the hub for the REACH and CLP Regulations, the new regulatory framework for chemical substances and mixtures in the European Union. REACH and CLP aim to improve the protection of human health and the environment while maintaining the competitiveness and enhancing the innovative capability of the EU chemicals industry.

ECHA is now recruiting the following staff:

Scientific Officers in Toxicology

The successful candidates will join a dynamic team engaged in establishing and operating the new regulatory framework, managing the technical, scientific and administrative aspects of REACH and CLP, ensuring consistency at Community level in its application and providing the Member States and the EU institutions with the best possible scientific and technical advice on chemicals.

They will work in ECHA's headquarters in Helsinki, Finland, where they will be employed as Temporary Agents under article 2 a) of Conditions of Employment of Other Servants of the European Communities. The deadline for applications is **28 February 2010**.

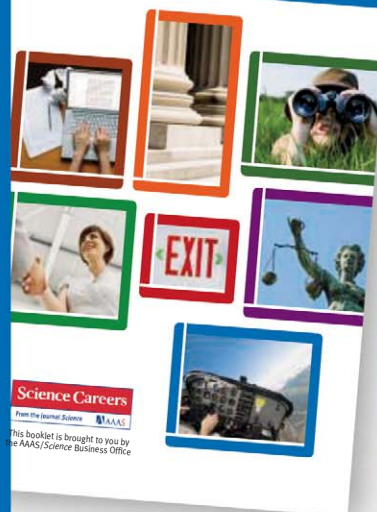
ECHA is an equal opportunities employer. For more details, please consult our job opportunities on the ECHA website: <http://www.echa.europa.eu>

<http://www.echa.europa.eu>

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Science Careers

From the journal *Science*



Publisher

Nature Publishing Group (NPG) is looking for an energetic Publisher to lead the Nature Physical Sciences publishing division into the future. This exciting opportunity will appeal to a professional with experience of managing journals and of developing and exploiting commercial opportunities.

The Nature Physical Sciences division brings together all of the Nature-branded journals in the Physical sciences - *Nature Materials*, *Nature Chemical Biology*, *Nature Physics*, *Nature Nanotechnology*, *Nature Geoscience*, *Nature Chemistry* and the soon to launch *Nature Climate Change*. The journals have enviable impact factors, a history of editorial excellence, and great commercial potential.

A key priority for the Publisher is to build on this base to develop the Nature Physical Sciences portfolio as it adapts to meet the changing needs of the market. The role calls for creative thinking, sound financial judgement, and an enlightened vision for the future. The Publisher will be expected to implement innovative programs to develop the portfolio, within the supportive and challenging environment of this world-leading scientific publishing company.

The successful candidate will directly manage the in-house editorial team comprising over 30 staff in London, Boston, San Francisco and Tokyo. The successful candidate will work closely with the Chief Editors and the Associate Publisher to develop new commercial opportunities for the journals. The Publisher will also focus on how the journals can best target the communities they serve through new technologies and enhancements to the existing journals. The role requires considerable creative flair as the journals move from 'print first' to 'online first' in their publishing strategies, and has great scope for experimentation and new business development.

The successful candidate will also be expected to develop a program of new products that will sit alongside the existing journals in the Nature Physical Sciences portfolio. Along with the Associate Publisher you will lead all stages of new product development - from creation of concepts, thorough market research and the generation of business plans to project-managing the launches. The Publisher will be expected to identify areas of opportunity and to ensure that plans are robust, achievable and financially sound.

Strong leadership, management skills and a track record in launching new products are essential for this role. The position will be based in London and, due to the international nature of the editorial teams, travel will be necessary.

The Publisher position is a senior appointment, and will report directly to the Publishing Director.

Applicants should provide, along with a CV and letter of application indicating salary expectation, a short document of 1000 to 1500 words outlining their strategy to develop a series of titles in the physical sciences in the next three to five years building on those already planned or published.

To apply please send your CV and covering letter quoting reference number NPG/005/10 to londonrecruitment@macmillan.co.uk

Closing date: 19th March 2010



School of Chemical and Mathematical Sciences

Shape the future of desalination research in Australia in one of the world's most liveable cities.

Applications are invited for the following positions associated with Australia's newly-established National Centre of Excellence in Desalination (NCED).

Based at Murdoch University's campus in Rockingham, Perth's major southern coastal suburb, the centre brings together the country's leading desalination and water science research organisations to lead and coordinate national and international research collaborations in energy-efficient desalination technology. The NCED has a budgeted A\$45M research portfolio and a national pilot scale testing and research facility currently in design.

With a population of 1.5 million people and a Mediterranean climate, Perth is one of the world's most liveable cities. It is the capital city of Western Australia, the nation's economic powerhouse, and it boasts beautiful beaches and a relaxed outdoors lifestyle in a very attractive, clean, spacious city.

Research Chair in Desalination / Chief Scientific Officer

Ref: 1382J17, Professor, Level E

Salary: A\$155k including superannuation and the opportunity to salary package. 48 months fixed term appointment.

We are looking for an experienced, passionate, senior researcher to provide the School and the NCED with visionary academic leadership, and take overall responsibility for the centre's research strategy and education program.

You will be a dynamic leader with a track record in managing collaborative research projects, ideally in the area of desalination. You will have personal eminence in innovative research and development, with well-established networks within international research and industry communities.

Lecturer, Level B (3 positions)

Ref: 0605E03, 0605E07, 0605E08

Salary Range: A\$83k to A\$100k including superannuation and the opportunity to salary package. 36 month fixed term contract from April 2010.

- Lecturer in Mineral Science
- Lecturer in Chemistry
- Lecturer in Applied Mathematics

High quality academics within these disciplines are encouraged to apply particularly if you have a strong research interest in your specialist area and have some alignment with aqueous solution processing, ideally desalination technology.

The School of Chemical and Mathematical Sciences, located on Murdoch's South Street campus, has a cross-disciplinary approach to research and teaching, with lecturing staff expected to contribute to undergraduate units taken by students from other Schools in the University.

As well as hosting the NCED, Murdoch University is a major partner in the Parker CRC for Integrated Hydrometallurgy Solutions, the primary research facility for hydrometallurgy in Australia. You will be expected to contribute significantly to the research activities of either or both of these centres.

For further information about these positions contact Professor Peter May by phone +61 8 9360 6068 or by email P.May@murdoch.edu.au

Application procedures and a position description are available from the University's web site at <http://jobs.murdoch.edu.au/>

Closing Date: Friday 26th March 2010

For more information about Perth visit www.westernaustralia.com



LYCERA CORP, a leading bio-pharmaceutical focused on the discovery and development of small-molecule immunomodulators for the treatment of patients with autoimmune diseases such as rheumatoid arthritis, psoriasis, and inflammatory bowel disease, has an opening for an IMMUNOLOGIST at its Ann Arbor campus. This individual will be engaged in studies to explore the mechanism of novel immunosuppressive agents, as well as the identification of new immunomodulatory pathways. Candidates must have a Ph.D., postdoctoral experience, and expertise in either lymphocyte or dendritic cell biology. Expertise in autoimmune disease or TH17 biology is desirable.

The successful candidate will possess a passion for and commitment to discovering and developing transformative, best in class therapeutics; strong desire to work in a scientifically rigorous and driven environment; and an entrepreneurial spirit who enjoys the autonomy that comes with this opportunity.

LYCERA offers a competitive salary, performance based bonus, stock options, and a comprehensive benefits and relocation package.

Interested individuals should submit curriculum vitae and the names of three references to the address below:

<i>Electronically</i>	<i>Paper</i>
LyceraJobs@lycera.com	Lycera Corp
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	Suite C
	Plymouth, MI 48170
	Attn: Human Resources

An Equal Opportunity Employer.



Northeastern College of Engineering Sternberg Professorship in Nanotechnology

The College of Engineering invites applications and nominations for the position of Sternberg Professor of Nanotechnology; an endowed chair within the College of Engineering. We seek renowned scholars who have achieved national and international distinction in fundamental and applied research in nanotechnology. The abilities to interact with industrial partners and to provide leadership to interdisciplinary research teams are essential. The successful candidate will be expected to establish a leading high-level research program, to teach at the graduate and undergraduate levels, and to interact with research groups at Northeastern. The college is interested in, but not limited to, the following topics: Nanomanufacturing, directed assembly, nanoscale devices, micro and nano robotics, single molecule and nanoscale devices, non-volatile switches, synthesis and functionalization of nanobuilding blocks, bio and chemical sensors.

Northeastern University is home to the NSF Nanoscale Science and Engineering Center for High-rate Nanomanufacturing (CHN: www.nano.neu.edu), one of four such centers nationwide. The CHN include more than 160 students, postdocs and professors. The CHN enjoys successful partnerships and collaborations with industry through its 36 member companies yielding a multi-million dollar annual research. The College of Engineering houses the George J. Kostas Nanoscale Technology and Manufacturing Research Center, a 10,000 ft² facility, including a 7,000 ft² Class 10/100 cleanroom, with a complete wafer nanofabrication facility. The College of Engineering has, in addition to the CHN, four other competitively funded major centers on subsurface sensing and imaging, detection of explosives, advanced sensors for infrastructure, and healthcare systems engineering.

Appointment will be at the tenured full and associate professor level and is expected to be made by Fall 2010. Nominations and applications, including a letter of interest, resume and references, should be sent to: **Professor Ahmed Busnaina, Chair, William Lincoln Smith Professor and Director, The NSF Nanoscale Science and Engineering Center for High-rate Nanomanufacturing, Northeastern University, 360 Huntington Avenue, Boston, MA 02115; Tel (617) 373-2992, Fax: (617) 373-2921; Email: busnaina@neu.edu.**

Northeastern is an Equal Opportunity/Affirmative Action, Title IX University.

march 8-9, 2010
Arizona Biltmore | Phoenix, Arizona



personalized medicine in the clinic:

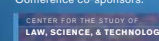
policy, legal, and ethical implications

This national conference with top experts will examine the impact of personalized medicine on the delivery of healthcare in the future. Conference highlights:

- patient rights
- medical privacy and confidentiality
- ethics
- individualized medical care
- economics
- liability issues for physicians

For CLE and CME information and to register, visit
www.law.asu.edu/personalizedmedicine2010.
To become a conference supporter, call 480.965.2465.

Conference co-sponsors:









JOHNS HOPKINS BLOOMBERG SCHOOL OF PUBLIC HEALTH

The Department of Environmental Health Sciences at the Johns Hopkins Bloomberg School of Public Health, invites applications for several faculty positions at the level of assistant professor, tenure-track. Successful applicants will be expected to develop and sustain a vigorous, extramurally funded research program in the field of environmental health sciences. The specific areas of research interest are expected to complement and enhance the wide range of expertise currently possessed by faculty in the department. In particular, candidates are encouraged to apply who have a research focus pertaining to: (1) the effects of exposure to environmental toxicants on development of neural, reproductive, or other organ systems; (2) specific environmental mediators of cardiopulmonary or metabolic disorders; (3) use of alternative models to study environmental diseases such as *C. elegans* or Zebrafish; (4) environmental epigenetics and genomics; (5) mechanisms of environmental based carcinogenesis or (6) molecular mechanisms of the effects of environmental exposures on signal transduction, immune system or other cellular processes. The Department offers excellent opportunities for cross cutting interdisciplinary research in areas including gene-environment interaction, environmental epidemiology, environmental and occupational health, toxicology, physiology and risk assessment for susceptibility to environmental diseases.

Successful applicants should have academic and research experience commensurate with the level of the appointment at the assistant professor level. In particular, they should have demonstrated a record of peer-reviewed publication and the potential to successfully obtain funding. Successful applicants will also be expected to contribute to the masters and doctoral education programs of the department.

Candidates should submit their curriculum vitae with names of three references, a statement summarizing their research experience and research plans sent to: **Christine Spangler, Search Committee Coordinator, Department of Environmental Health Sciences, The Johns Hopkins University Bloomberg School of Public Health, 615 N. Wolfe St, Baltimore, MD 21205** or candidates may submit application materials in portable document format (PDF) via e-mail: cspangle@jhsph.edu. Only complete applications will be reviewed.

35TH FEBS CONGRESS MOLECULES OF LIFE

Welcome to FEBS 2010, jointly organised by the
Swedish Society for Biochemistry and Molecular
Biology and the Norwegian Biochemical Society

June 26–July 1, 2010 • Gothenburg, Sweden • at Gothenburg Convention Centre (Svenska Mässan)

Programme highlights

Nobel Laureate lectures: Roger Tsien (UCSD; Nobel Prize 2008)
Venki Ramakrishnan (MRC-LMB; Nobel Prize 2009)
Elizabeth Blackburn (UCSF; Nobel Prize 2009)
John Walker (MRC-MBU; Nobel Prize 1997)
Datta lecture: Juleen Zierath (Karolinska Institute)
Krebs lecture: Harald Stenmark (Norwegian Radium Hospital)
Bücher lecture: Svante Pääbo (MPI Leipzig)
EMBO lecture: Uri Alon (Weizmann Institute)
IUBMB lecture: Susan Lindquist (Whitehead Institute)

Congress symposia

- A - Molecules in Health and Disease**
- B - Molecular Networks**
- C - Molecules at Work**
- D - Cellular Compartments**
- E - Biomolecular Design and Function**

Important dates

Early registration	February 26, 2010
Deadline for support application	February 26, 2010
Abstract deadline	March 31, 2010
Congress starts	June 26, 2010 at 17.00

Workshops on technology developments

- Sequencing technology
- Proteomics technologies
- Metabolomics
- Protein structures
- Life imaging
- Dynamic modelling
- Protein interactions
- Molecular imaging
- Protein expression
- Lipidomics
- Network modelling
- Bioinformatics

Activities by FEBS committees and working groups

- Science & Society
- Education
- Women in Science



YOUNG SCIENTIST FORUM
LIFE OF MOLECULES
June 23-26 2010 Gothenburg

www.febs2010.org

ANNOUNCEMENTS

FUNDING OPPORTUNITY

**James S. McDonnell
Foundation**

**21st Century Science
Initiative 2010**

Research Award Application

Deadline: 15:59 CDT on

Wednesday, March 17, 2010

No geographic restrictions; international applications are encouraged.

Updated program descriptions and application guidelines for 21st Century Science Initiative Research Awards supporting investigator initiated research in two (2) topical areas: Studying Complex Systems and Brain Cancer Research are now available at:
www.jsmf.org/apply/research.

For information about the McDonnell Foundation including lists of grants funded in 2009, visit our website: www.jsmf.org.



**LEHIGH
UNIVERSITY**

Presidential Endowed Chair: Professorship in Health-related Sciences/Engineering Search

Lehigh University is seeking applications and nominations for a Presidential Endowed Professorship in Health in the Natural Sciences or Engineering fields as an important component of a university-wide health initiative. We encourage applications from established scholars open to cross-disciplinary collaboration and whose research activities intersect with those of existing groups at Lehigh University. We offer a uniquely supportive environment for cross-disciplinary research over a broad range of fields, and we seek a dynamic individual who would thrive in such an environment and could serve as a catalyst for new cross-cutting research collaborations. Candidates for the Presidential Chair must have a distinguished record in health-related research in the natural sciences or engineering disciplines and a record of external funding to support their research endeavors.

Appointment, at the Associate or Full Professor rank, will include a competitive salary and start-up package, and will be in the appropriate disciplinary department. A concurrent search is also underway in the social sciences and humanities. Additional information about the health initiative is available at <http://www.lehigh.edu/healthsearches/scienceandengineering/>.

Applications should be sent by email to presidentialchairhealthscieng@lehigh.edu. Please include a cover letter, CV, and the names of three potential references.

Nominations are also welcome, and will be reviewed and contacted by the Search Committee. Names of potential candidates can be sent to presidentialchairhealthscieng@lehigh.edu.

Review of applications will be ongoing until the position is filled.

POSITIONS OPEN



汕头大学医学院
Shantou University Medical College

OPENINGS FOR CLINICAL TEACHING FACULTY IN CHINA

Several full- and part-time (greater than three months) positions are available for experienced professionals to teach clinical as well as preclinical courses at Shantou University Medical College, Guangdong Province, China. Successful candidates will teach in our English-based Integrated Medical Curriculum, which will include didactic lectures, problem-based learning activities, small-group discussions, laboratory exercises, and/or community-based activities in all disciplines. They will work side-by-side with our dedicated faculty and coach our students into becoming skillful physicians with advanced international standards. The opportunity will be stimulating and rewarding both academically and culturally, and the school will provide a comfortable and friendly environment with a competitive package. Please submit documents or inquiries electronically to **Professor William W. Au, Associate Dean of the four-year English medical curriculum, e-mail: wau@stu.edu.cn**. The documents should include a brief biography, curriculum vitae, a personal statement (includes teaching expertise, availability, and a list of three references). We have a preference for candidates with M.D. degrees and encourage recently retired professors to apply. Knowledge of the Chinese language is not necessary.

ENDOWED CHAIR, MOLECULAR INSECT PHYSIOLOGY. Purdue University invites qualified applicants to apply for the Endowed Chair position in insect molecular physiology, Department of Entomology. Application review will begin March 1, 2010. See detailed announcement at **website: <http://www.ag.purdue.edu/entm/Pages/rollins-orkin.aspx>**. Submit inquiries and applications to **e-mail: yanineck@purdue.edu**. *Purdue University is an Equal Opportunity/Equal Access/Affirmative Action Employer.*

ANNOUNCEMENT



STANFORD
SCHOOL OF MEDICINE

STANFORD BIOCHEMISTRY FOUNDERS AWARD FOR DOCTORAL EXCELLENCE

We seek nominations for the second annual Stanford Biochemistry Founders Award to recognize outstanding achievement by doctoral scholars as part of our commitment to advancing gender diversity in biochemistry and molecular biosciences.

Recipients will participate in a one-day symposium in May 2010 at Stanford University. The symposium will consist of scientific presentations by the awardees and by Stanford faculty, and informal discussions with students and faculty. Awardees will be advanced students near the completion of their studies and will not have graduated before September 1, 2009. Up to four awardees will be selected on the basis of the quality, originality, and significance of their work; the award will include travel and accommodation expenses and a \$500 honorarium.

Nominations should be submitted electronically (as a single PDF document) by a faculty member, and should include the student's curriculum vitae, a one-page description of the thesis work (written by the student), and a recommendation letter. A second recommendation letter (PDF) should be sent separately by its author. Nomination materials should state clearly how the nominee's work has advanced our understanding of the molecular basis of a significant biological process, as well as how this award will help to advance gender diversity in the field.

The submission deadline is March 20, 2010; send electronically to **e-mail: cspitale@stanford.edu**.

POSITIONS OPEN



Stanford Molecular Imaging
Scholars (SMIS) Program

POSTDOCTORAL TRAINING in Molecular Imaging of Cancer

Stanford University, Stanford, CA, U.S.A.
Principal Investigator: Sanjiv Sam Gambhir, M.D., Ph.D.

The Stanford Molecular Imaging Scholars (SMIS) Program is a diverse training program bringing together more than 13 departments, predominantly from the Stanford Schools of Medicine and Engineering, in order to train the next generation of interdisciplinary leaders in molecular imaging. Within molecular imaging, oncologic molecular imaging is a rapidly growing area, which combines the disciplines of chemistry, cell/molecular biology, molecular pharmacology, physics, bioengineering, imaging sciences, and clinical medicine to advance cancer research, diagnosis, and management. SMIS fellows will conduct innovative research in cancer imaging under the supervision of two faculty mentors from complementary fields, in a comprehensive, integrated, flexible program (up to three years). Funding is guaranteed for one year, with the possibility of a second and third year.

(1) Application deadline: May 10, 2010, for a start date in September 2010. (2) Applicants must have a Ph.D. or M.D. and *must be U.S. citizens or permanent residents*. (3) For more information, see **website: <http://mips.stanford.edu/smis/>**.

Inquiries to **Sofia Gonzales, telephone: 650-724-9139; e-mail: sofias@stanford.edu**.

BASIC SCIENCE PRINCIPAL INVESTIGATOR, RESEARCH CORE DIRECTOR

Hampton University
Skin of Color Research Institute

The Hampton University Skin of Color Research Institute (HUSCRI), a newly established research institute, is seeking an outstanding principal investigator to assume leadership of the HUSCRI research program as its Principal Investigator, Research Core Director. The successful candidate will be hired at the rank of **ASSISTANT or ASSOCIATE PROFESSOR** with experience in the area of cutaneous biology and specific interests in wound healing or pigment cell research. He/She will be expected to create and lead an innovative and aggressive research strategy that is harmonious with the mission and vision of Hampton University. He/She will have the opportunity to initiate, foster, and grow collaborative relationships with investigators and neighboring institutions including Eastern Virginia Medical School and Thomas Jefferson National Accelerator Facility. Newly outfitted HUSCRI laboratories will be housed in a 20,000-square-foot biomedical research building on the campus of Hampton University.

Requirements: (1) Successful candidates must have either a Ph.D. or M.D.-Ph.D.; (2) Strong publication record in peer reviewed journals; (3) Record of demonstrated leadership; (4) History of success in attaining federally funded grants in areas of interest preferred.

Review of applications will begin immediately and will continue until the position is filled. The compensation package will be commensurate with experience. Interested candidates should submit their curriculum vitae, a synopsis of their past and ongoing research projects and accomplishments, personal contact information, and names of three professional references along with postal and e-mail addresses and telephone numbers electronically to **e-mail: sharon.gillis@hamptonu.edu** or by surface mail to:

Dr. Pamela V. Hammond
Provost and Chairman of Search Committee
Office of the Provost
Hampton University
Hampton, VA 23668

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POSITIONS OPEN

THE UNIVERSITY of TENNESSEE
HEALTH SCIENCE CENTER

POSTDOCTORAL POSITIONS. Two positions are immediately available to study the role of lipoxigenases/eicosanoids and chemokines in angiogenesis and vascular wall remodeling. Experience with animal models of angiogenesis, restenosis and/or molecular cloning is highly desirable. Based on experience, competitive salaries are offered. Interested and highly motivated candidates with Ph.D., M.D., or M.D.-Ph.D. degree should send curriculum vitae and names and addresses of three references to: **GN Rao, Ph.D., Department of Physiology, University of Tennessee Health Science Center, 894 Union Avenue, Memphis, TN 38163. E-mail: rgadipar@uthsc.edu**. *The University of Tennessee is an Equal Employment Opportunity/Affirmative Action Title VI/Title IX/Section 504/ADA/ADEA Institution in the provision of its education and employment programs and services.*

FACULTY POSITION

Florida Institute of Technology
Department of Biological Sciences

Florida Institute of Technology (**website: <http://www.fit.edu>**), an independent, technological university located on Florida's east coast, invites applications for an **ASSISTANT or ASSOCIATE PROFESSOR** position beginning in fall 2010. Candidates must have expertise in plant biology and biochemistry, a Ph.D. degree, and relevant postdoctoral experience. Successful candidates are expected to participate in undergraduate and graduate (M.S./Ph.D.) teaching, and will be expected to establish an active, externally funded research program. We also seek a colleague who will support our growing M.S. biotechnology program. Review of applications will commence immediately. Interested candidates should submit a cover letter, detailed curriculum vitae, statements of research interests and teaching philosophy, and contact information for three references to:

Prof. Alan C. Leonard
Department of Biological Sciences
Florida Institute of Technology
150 W. University Boulevard
Melbourne, FL 32901-6975
E-mail: **aleonard@fit.edu**

Equal Opportunity/Affirmative Action/ADA Compliant Employer. Women and minorities are strongly encouraged to apply.

POSTDOCTORAL POSITION

Department of Anatomy and Regenerative Biology
George Washington University Medical Center
Washington, D.C.

NIH-funded position available immediately to use biocytin injections and confocal imaging to identify the inputs of brainstem vestibular nuclei. Ph.D. or M.D. required and a strong background in neurobiology with publications. Send curriculum vitae electronically to **Dr. Kenna Peusner, e-mail: anakdp@gwumc.edu**. *The George Washington University is an Equal Opportunity/Affirmative Action Employer. Women and minorities are encouraged to apply.*

Translational Neuroscience. The University of Arizona seeks to hire a tenure-eligible physician-scientist at the **ASSISTANT or ASSOCIATE** level in neuroscience. Visit **website: <https://www.uacaretrack.com>** or electronically contact **Search Committee Chair, Carol A. Barnes, Ph.D., e-mail: carol@nsma.arizona.edu**. *Affirmative Action/Equal Opportunity Employer.*

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Papers in the following areas will be reviewed and considered for publication:

- Animal & Human Studies
- Applied Physical Sciences
- Behavior
- Bioengineering
- Biomarkers
- Cancer
- Cardiovascular Disease
- Cell Culture
- Chemical Genomics/Drug Discovery
- Data Mining
- Drug Delivery
- Gene Therapy/Regenerative Medicine
- Imaging
- Immunology/Vaccines
- Infectious Diseases
- Medical Informatics
- Medical Nanotechnology
- Metabolism/Diabetes/Obesity
- Neuroscience/Neurology/Psychiatry
- Pharmacogenetics
- Policy
- Toxicology & Pharmacokinetics
- And other interdisciplinary approaches to medicine



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